Supporting Information

Synthesis of *seco*-B-Ring Bryostatin Analogue WN-1 *via* C-C Bond Forming Hydrogenation: Critical Contribution of the B-Ring in Determining Bryostatin- and PMA-Like Properties

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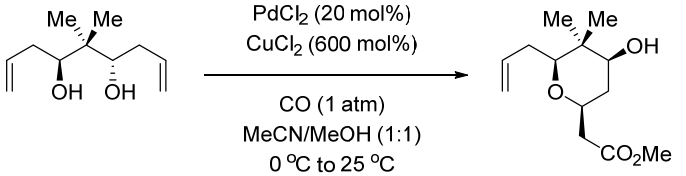
General Methods

All reactions were run under an atmosphere of argon, under anhydrous conditions unless otherwise stated. All glassware was dried in an oven overnight and cooled under a stream of nitrogen prior to use. Dichloroethane (DCE) and dichloromethane (DCM) were freshly distilled from calcium hydride immediately prior to use. All commercially available reagents and solvents were purchased and used as received unless otherwise stated here. Anhydrous Copper (II) chloride was prepared from the hydrate by heating to 100 °C under vacuum (The green hydrate turns brown in color upon dehydration). Large batches of anhydrous copper (II) chloride were prepared in this manner and stored in the absence of moisture for future use. Triethylamine and diisopropylethylamine were distilled from potassium hydroxide prior to use. Tetrahydrofuran (THF), diethyl ether, 1,4-dioxane, and toluene were freshly distilled from sodium benzophenone ketyl immediately prior to use. All TLC analyses were carried out on Silicycle SiliaPlate glass backed TLC plates (250 μM thickness) with F-254 indicator. Visualization was carried out with UV light, by dipping in *p*-anisaldehyde stain followed by heating, or by dipping in potassium permanganate stain followed by heating. Flash column chromatography was carried out using Silicycle SiliaFlash P60 silica gel (230 – 400 mesh). All infrared (IR) spectra were recorded using a Nicolet 380 FTIR instrument. High resolution mass spectrometry (HRMS) data was collected on an Agilent Technologies 6530 Accurate Mass Q-Tof LC/MS instrument. Proton and carbon NMR data was collected on Varian DirectDrive 400 MHz, Agilent MR 400 MHz, Varian INOVA 500 MHz, and Varian DirectDrive 600 MHz spectrometers. Proton and carbon NMR chemical shifts are reported in parts per million (ppm) relative to the residual solvent peak ($CDCl_3$ in all cases, 7.26 for ^{1}H and 77.0 for ^{13}C). Coupling constants are reported in Hertz (Hz). Specific rotations were measured using an Atago AP-300 automatic polarimeter and are reported as the average value of three measurements.

I. Detailed Procedures and Spectral Data for Compounds



4 **5**

73% Yield, >20:1 dr

To a round bottom flask containing a magnetic stir bar was added palladium (II) chloride (193 mg, 1.09 mmol, 20 mol%) and copper (II) chloride (4.38 g, 32.6 mmol, 600 mol%). The flask was placed under vacuum and replenished with carbon monoxide three times then capped with a septum fitted with a balloon of CO. Acetonitrile (26 mL) was added and the heterogeneous mixture is stirred vigorously until a fine, even suspension is obtained (Note: it is important to generate a fine suspension at this point as it was found that proceeding with the reaction before this is achieved often led to undesired side-product formation). The suspension was cooled to 0 °C and a solution of diol **4**[1] (1g, 5.43 mmol, 100 mol%) in methanol (26 mL) was added over a few minutes. The reaction was stirred at 0 °C for 20 minutes then allowed to warm to room temperature and stir for an additional 90 minutes. The solvent was removed *in vacuo* and the salts were dissolved in saturated $NH_4Cl_{(aq)}$. The aqueous phase was extracted three times with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified via flash column chromatography (25% EtOAc/Hex) to yield pyran **5** (961 mg, 73%) as a yellow oil.

Characterization data for pyran **5**

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (dddd, *J* = 17.3, 10.2, 7.2, 6.0 Hz, 1H), 5.11 – 4.87 (m, 2H), 3.92 – 3.71 (m, 1H), 3.66 (s, 3H), 3.42 (dd, *J* = 11.6, 4.7 Hz, 1H), 2.98 (dd, *J* = 9.8, 2.7 Hz, 1H), 2.58 (dd, *J* = 15.0, 8.1 Hz, 1H), 2.41 (dd, 15.0, 5.3 Hz, 1H), 2.29 – 1.99 (m, 2H), 1.77 (ddd, *J* = 12.4, 4.7, 2.3 Hz, 1H), 1.68 (br s, 1H), 1.42 (dt, *J* = 12.5, 11.6 Hz, 1H), 0.93 (s, 3H), 0.82 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.52, 136.33, 115.32, 83.60, 75.04, 72.45, 51.45, 40.72, 38.56, 36.24, 33.06, 22.15, 12.26.

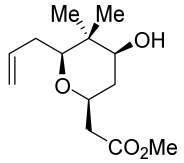
FTIR (neat) υ 3414, 2950, 2869, 1732, 1641, 1438, 1386, 1256, 1214, 1177, 1142, 1080 cm^{-1}.

HRMS (ESI) Calcd. for $C_{13}H_{22}O_4Na$ $[M+Na]^+$: 265.1416, Found: 265.1412.

$[\alpha]^{22.5}_D$: -58.33° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.24 (30% ethyl acetate in hexanes).

[1] (a) Lu, Y.; Kim, I.S.; Hassan, A.; Del Valle, D. J.; Krische, M. J. *Angew. Chem., Int. Ed.* **2009**, *48*, 5018. (b) Waldeck, A. R.; Krische, M. J. *Angew. Chem. Int. Ed.* **2013**, *52*, 4470.



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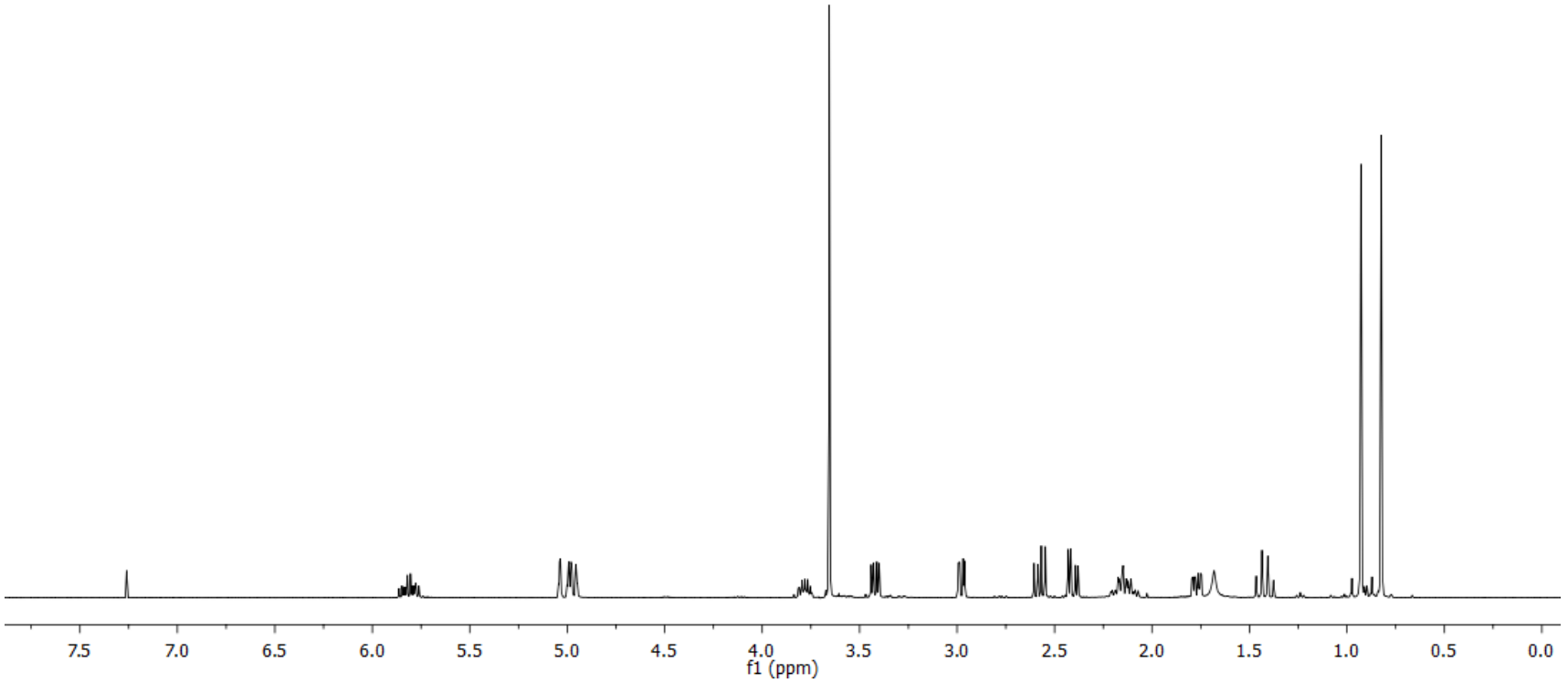

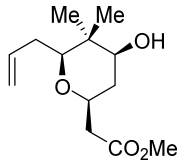
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O
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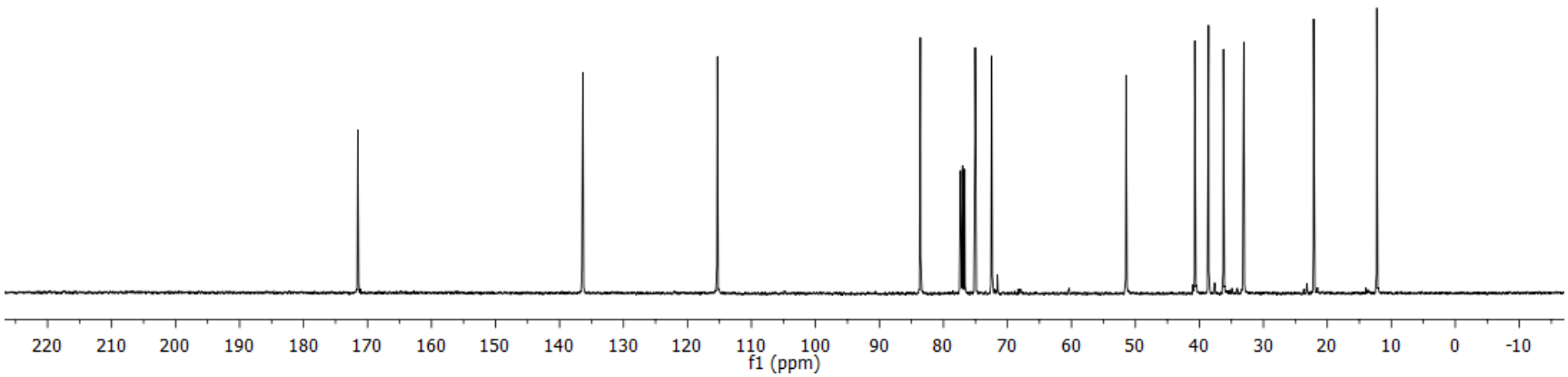
5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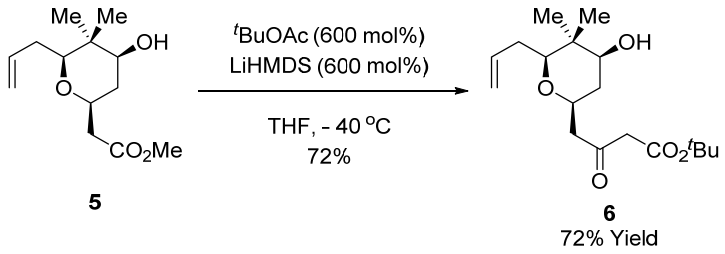
10
0
-10
f1 (ppm)



A round bottom flask equipped with a stir bar was placed under argon and charged with THF (42 mL) and LiHMDS (50.2 mL of a 1M solution in THF, 50.2 mmol, 600 mol%). The mixture was cooled to -40 °C and a solution of *t*-BuOAc (6.77 ml, 50.2 mmol, 600 mol%) in THF (13.5 mL) was added dropwise over ~15 minutes. The reaction was stirred at -40 °C for 40 minutes at which point a solution of pyran **5** (2.02 g, 8.34 mmol, 100 mol%) in THF (42 mL) was added dropwise over ~30 minutes. The reaction was stirred at -40 °C for 2.5 hours. The reaction was quenched while still at -40 °C by the addition of saturated $NH_4Cl_{(aq)}$ followed by warming to room temperature. The aqueous phase was extracted five times with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash column chromatography (10%→15%→20% EtOAc/Hex) to yield ketoester **6** (1.95 g, 72%) as a yellow oil.

Characterization data for ketoester **6**

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (dddd, *J* = 17.2, 10.1, 7.1, 6.3 Hz, 1H), 5.08 – 4.97 (m, 2H), 3.80 (dddd, *J* = 11.5, 8.1, 4.6, 2.4 Hz, 1H), 3.46 – 3.38 (m, 3H), 2.98 (dd, *J* = 10.1, 2.4 Hz, 1H), 2.78 (dd, *J* = 15.4, 8.1 Hz, 1H), 2.55 (dd, *J* = 15.4, 4.6 Hz, 1H), 2.25 – 2.16 (m, 1H), 2.16 – 2.06 (m, 1H), 1.77 (ddd, *J* = 12.5, 4.7, 2.4 Hz, 1H), 1.60 (br s, 1H), 1.49 – 1.39 (m, 10H), 0.94 (s, 3H), 0.83 (s, 3H).

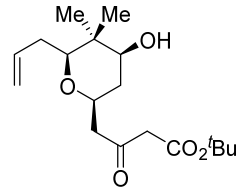
^{13}C NMR (100 MHz, $CDCl_3$): δ 201.9, 166.5, 136.5, 116.0, 83.8, 81.8, 75.3, 72.4, 51.4 48.7, 38.8, 36.6, 33.4, 28.0, 22.3, 12.4.

FTIR (neat) υ 3471, 2976, 2934, 2871, 1711, 1642, 1471, 1368, 1323, 1252, 1147, 1096 cm^{-1}.

HRMS (ESI) Calcd. for $C_{18}H_{30}O_5Na$ $[M+Na]^+$: 349.1991, Found: 349.1994.

$[\alpha]^{20.9}_D$: -51.22° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.18 (30% ethyl acetate in hexanes).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Me
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O
CO2tBu
O

6

7.5
7.0
6.5
6.0
5.5
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3.0
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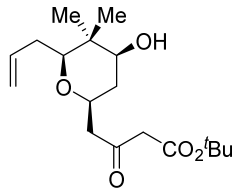
f1 (ppm)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CO2tBu
O

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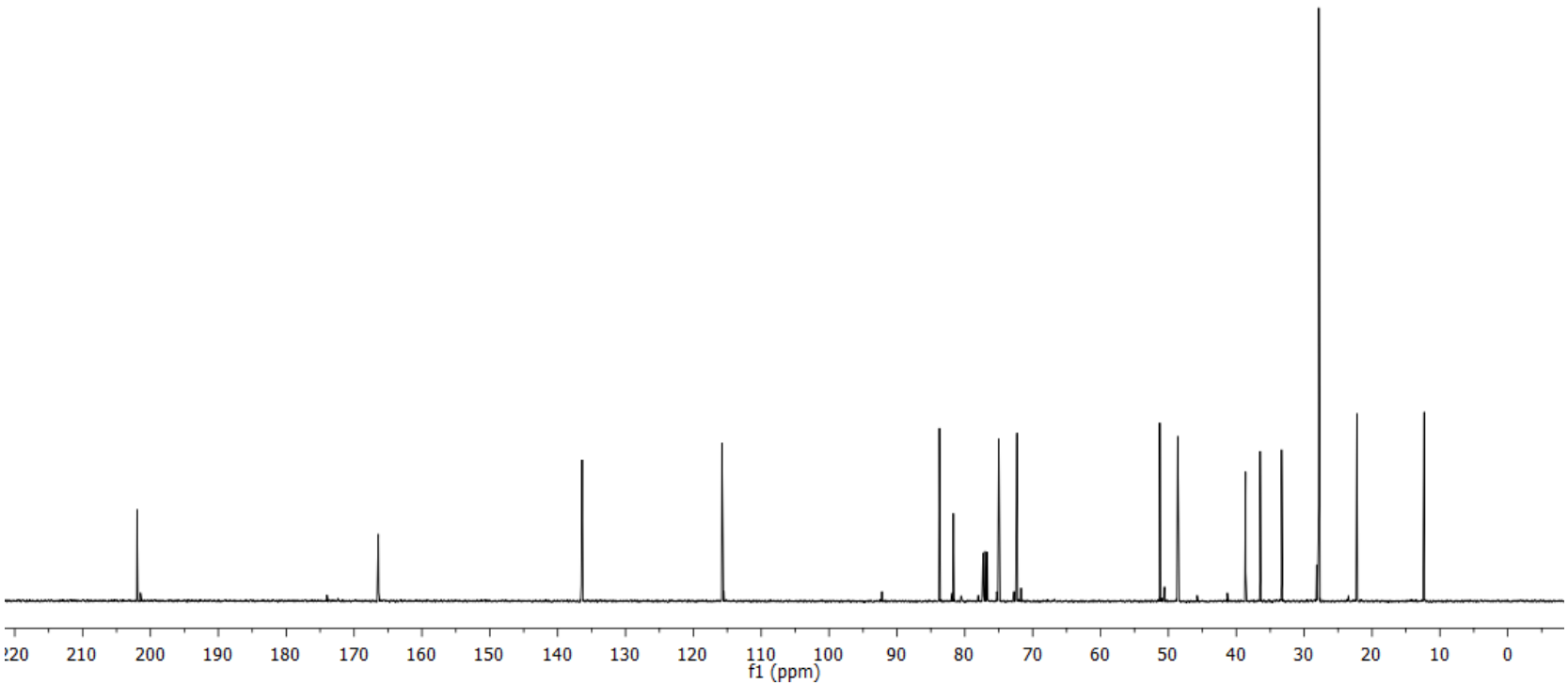
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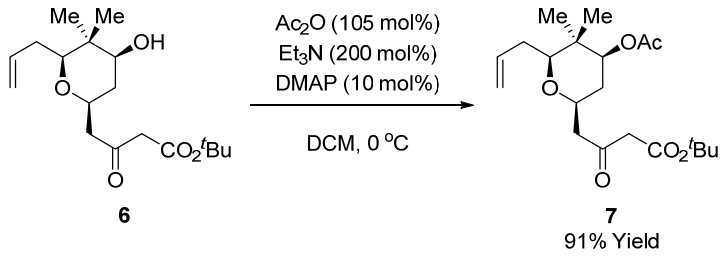
f1 (ppm)



6 **7**
91% Yield

To a round bottom flask containing alcohol **6** (905 mg, 2.77 mmol, 100 mol%) and dichloromethane (18.5 mL) was added DMAP (34 mg, 0.28 mmol, 10 mol%) and triethylamine (770 µL, 5.54 mmol, 200 mol%). The solution was put under argon and cooled to 0 °C. Acetic anhydride (270 µL, 2.91 mmol, 105 mol%) was added dropwise. The reaction was stirred at 0 °C for 16 hours then quenched by the addition of saturated $NaHCO_{3(aq)}$. The layers were separated and the aqueous phase extracted twice with dichloromethane. The combined organics were washed with brine, dried over Na_2SO_4 and concentrated. The crude residue was purified via flash column chromatography (10% EtOAc/Hex) to yield acetate **7** (930 mg, 91%) as a yellow oil.

Characterization data for acetate **7**

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (dddd, *J* = 17.2, 10.2, 7.1, 6.3 Hz, 1H), 5.10 – 4.97 (m, 2H), 4.66 (dd, *J* = 11.6, 4.8 Hz, 1H), 3.85 (dddd, *J* = 11.6, 8.3, 4.3, 2.4 Hz, 1H), 3.41 (d, *J* = 0.8 Hz, 2H), 3.08 (dd, *J* = 10.0, 2.5 Hz, 1H), 2.75 (dd, *J* = 15.3, 8.2 Hz, 1H), 2.55 (dd, *J* = 15.3, 4.3 Hz, 1H), 2.25 – 2.09 (m, 2H), 2.05 (s, 3H), 1.83 (ddd, *J* = 12.4, 4.8, 2.4 Hz, 1H), 1.51 – 1.43 (m, 10H), 0.92 (s, 3H), 0.84 (s, 3H).

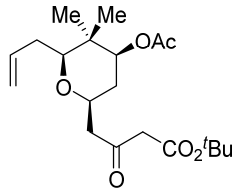
^{13}C NMR (100 MHz, $CDCl_3$) δ 201.3, 170.4, 166.3, 136.0, 116.1, 83.7, 81.6, 76.6, 72.0, 51.2, 48.4, 37.5, 33.2, 28.2, 27.8, 22.2, 21.0, 13.5.

FTIR (neat) υ 2976, 2935, 2874, 1739, 1718, 1663, 1643, 1392, 1367, 1241, 1196, 1148, 1118, 1025 cm^{-1}.

HRMS (ESI) Calcd. for $C_{20}H_{32}O_6Na$ $[M+Na]^+$: 391.2097, Found: 391.2091.

$[\alpha]^{29.0}_D$: -9.67° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.13 (10% ethyl acetate in hexanes).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O
CO2tBu
O

7

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6.0
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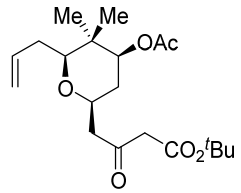
f1 (ppm)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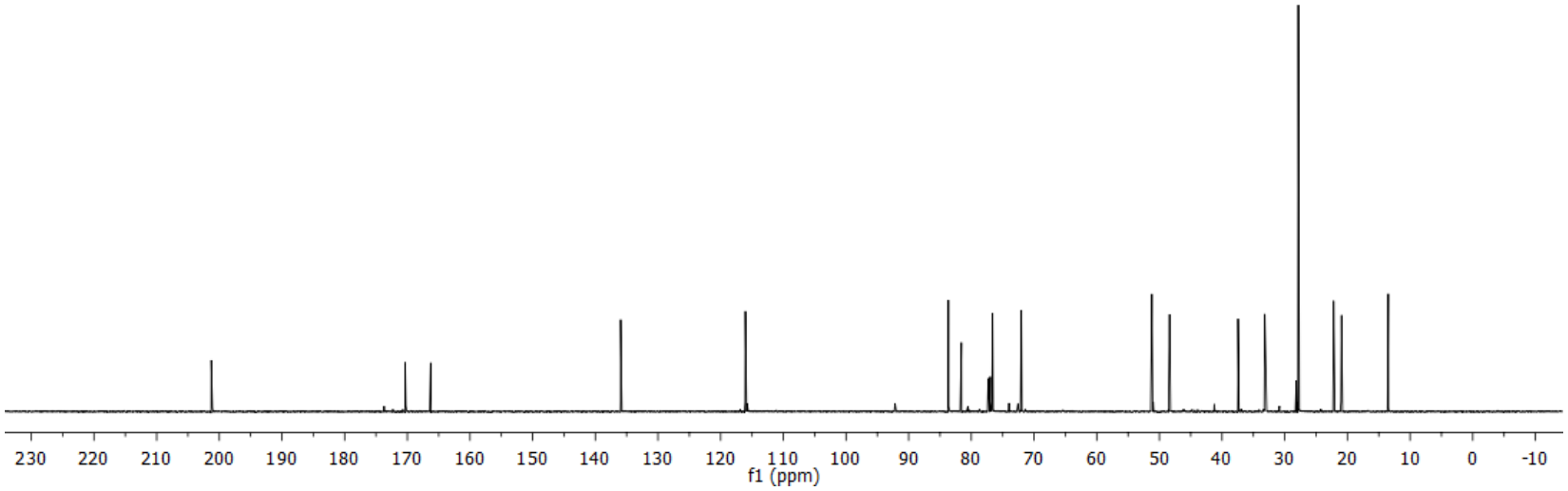
7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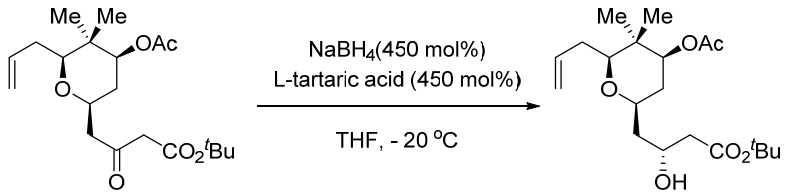


7 **8**

65% Yield, 9:1 dr

A round bottom flask was charged with $NaBH_4$ (397 mg, 10.5 mmol, 450 mol%) and put under argon. THF (26.2 mL) was added followed by L-tartartic acid (1.57 g, 10.5 mmol, 450 mol%). The heterogeneous slurry was stirred at 65 °C for three hours then allowed to cool to room temperature. The slurry was then cooled to – 20 °C and ketoester **7** (860 mg, 2.34 mmol, 100 mol%) was added dropwise as a solution in THF (4.5 mL). The reaction was stirred at – 20 °C for 48 hours then quenched by careful addition of 3M $HCl_{(aq)}$ (20 mL). The aqueous phase was extracted three times with ethyl acetate. The combined organic extracts were washed with saturated $NaHCO_{3(aq)}$ then brine. The organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified via flash column chromatography (10% → 20% EtOAc/Hexanes) to yield alcohol **8** (562 mg, 65%) as a slightly colored oil.

Characterization data for alcohol **8**

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.12 – 5.02 (m, 2H), 4.66 (dd, *J* = 11.6, 4.8 Hz, 1H), 4.21 (td, *J* = 7.6, 4.1 Hz, 1H), 3.75 – 3.67 (m, 1H), 3.34 (d, *J* = 4.9 Hz, 1H), 3.11 (dd, *J* = 10.2, 2.4 Hz, 1H), 2.48 – 2.34 (m, 2H), 2.28 – 2.19 (m, 1H), 2.18 – 2.07 (m, 1H), 2.06 (s, 3H), 1.75 (ddd, *J* = 12.5, 4.8, 2.4 Hz, 1H), 1.69 – 1.62 (m, 2H), 1.59 – 1.48 (m, 1H), 1.45 (s, 10H), 0.93 (s, 3H), 0.85 (s, 3H).

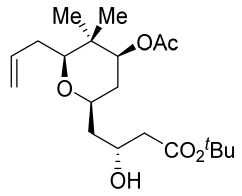
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 170.5, 136.0, 116.4, 83.5, 80.8, 77.1, 72.9, 65.6, 42.6, 41.3, 37.6, 33.6, 33.3, 28.0, 22.3, 21.1, 13.6.

FTIR (neat) υ 3523, 2976, 2932, 1724, 1392, 1367, 1242, 1152, 1089, 1026, 990, 911, 732 cm^{-1}.

HRMS (ESI) Calcd. for $C_{20}H_{34}O_6Na$ $[M+Na]^+$: 393.2253, Found: 393.2237.

$[\alpha]^{25.0}_D$: -13.3° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.36 (30% ethyl acetate in hexanes).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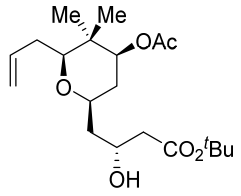
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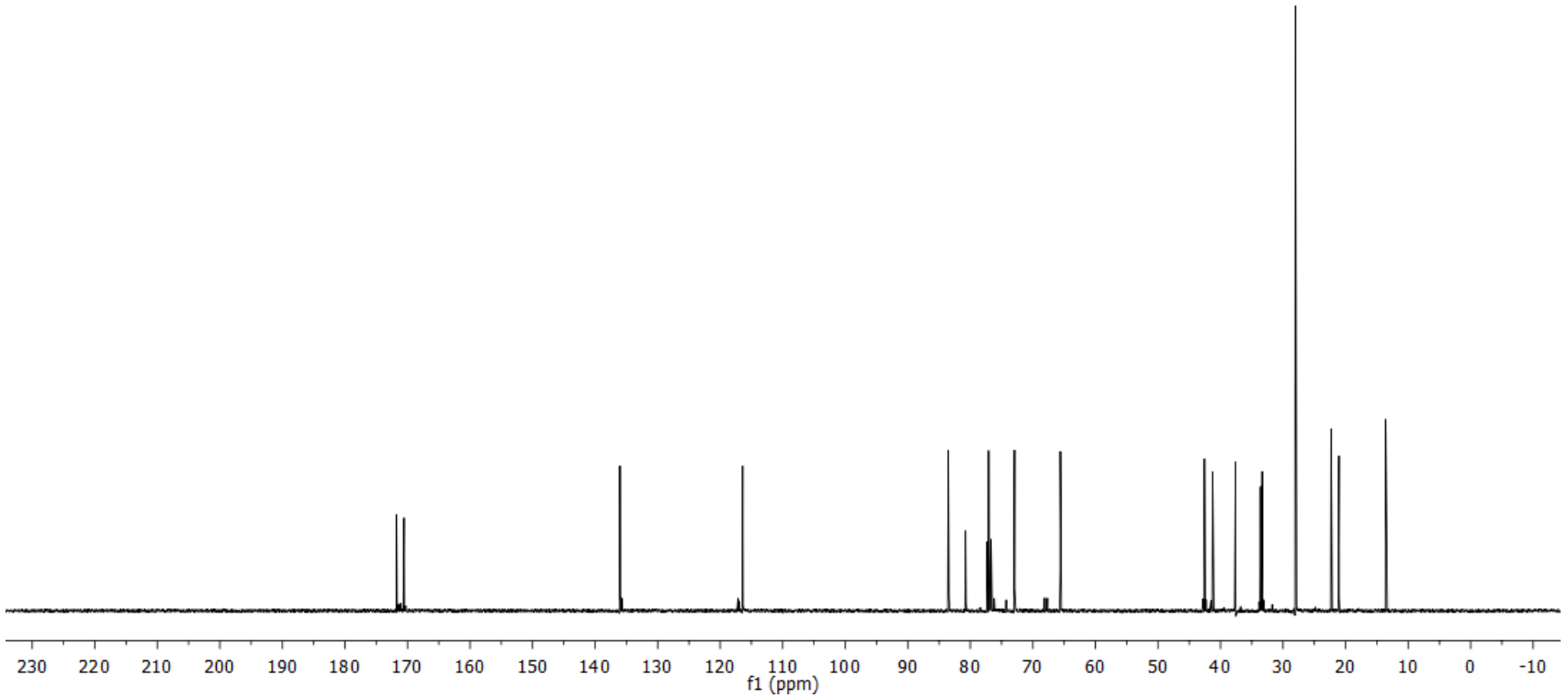
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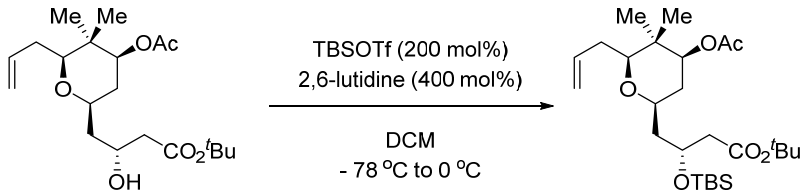


Alcohol **8** (868 mg, 2.3 mmol) was dissolved in dichloromethane (24 mL) and put under an argon atmosphere. 2,6-lutidine (1.1 mL, 9.4 mmol, 400 mol%) was added and the reaction was cooled to – 78 °C. TBSOTf (1.1 mL, 4.6 mmol, 200 mol%) was added and the reaction was stirred at – 78 °C for ten minutes then transferred to a bath at 0 °C. The reaction was stirred at 0 °C for 30 minutes then quenched with water (8 mL). The layers were separated and the aqueous phase was extracted three times with dichloromethane. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified via flash column chromatography (5% EtOAc/Hexanes) to yield TBS-ether **9** (905 mg, 80%) as a yellow oil.

Characterization data for TBS-ether **9**

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (dddd, *J* = 17.3, 10.1, 7.3, 6.6 Hz, 1H), 5.04 (ddt, *J* = 17.1, 2.1, 1.4 Hz, 1H), 4.99 (ddt, *J* = 10.0, 2.1, 1.1 Hz, 1H), 4.64 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.25 (dtd, *J* = 8.3, 6.0, 3.7 Hz, 1H), 3.50 (ddt, *J* = 11.9, 9.6, 2.5 Hz, 1H), 3.01 (dd, *J* = 9.7, 2.8 Hz, 1H), 2.46 – 2.28 (m, 2H), 2.27 – 2.08 (m, 2H), 2.05 (s, 3H), 1.76 – 1.61 (m, 2H), 1.55 (ddd, *J* = 14.2, 8.4, 2.5 Hz, 1H), 1.43 (d, *J* = 1.1 Hz, 9H), 0.90 (s, 3H), 0.86 (s, 9H), 0.83 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H).

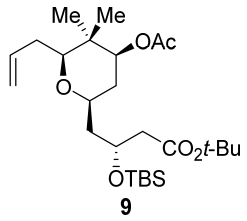
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.6, 136.6, 116.1, 83.3, 80.1, 77.3, 71.9, 66.2, 44.9, 44.0, 37.7, 34.2, 33.8, 28.1, 25.8, 22.4, 21.1, 17.9, 13.6, -4.5, -4.7.

FTIR (neat) υ 2954, 2930, 2857, 1732, 1643, 1472, 1391, 1366, 1242, 1159, 1081 cm^{-1}.

HRMS (ESI) Calcd. for $C_{26}H_{48}O_6SiNa$ $[M+Na]^+$: 507.3138, Found: 507.3106.

$[\alpha]^{25.0}_D$: -6.15° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.38 (10% ethyl acetate in hexanes).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9

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3.5
3.0
2.5
2.0
1.5
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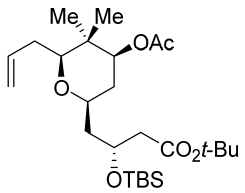
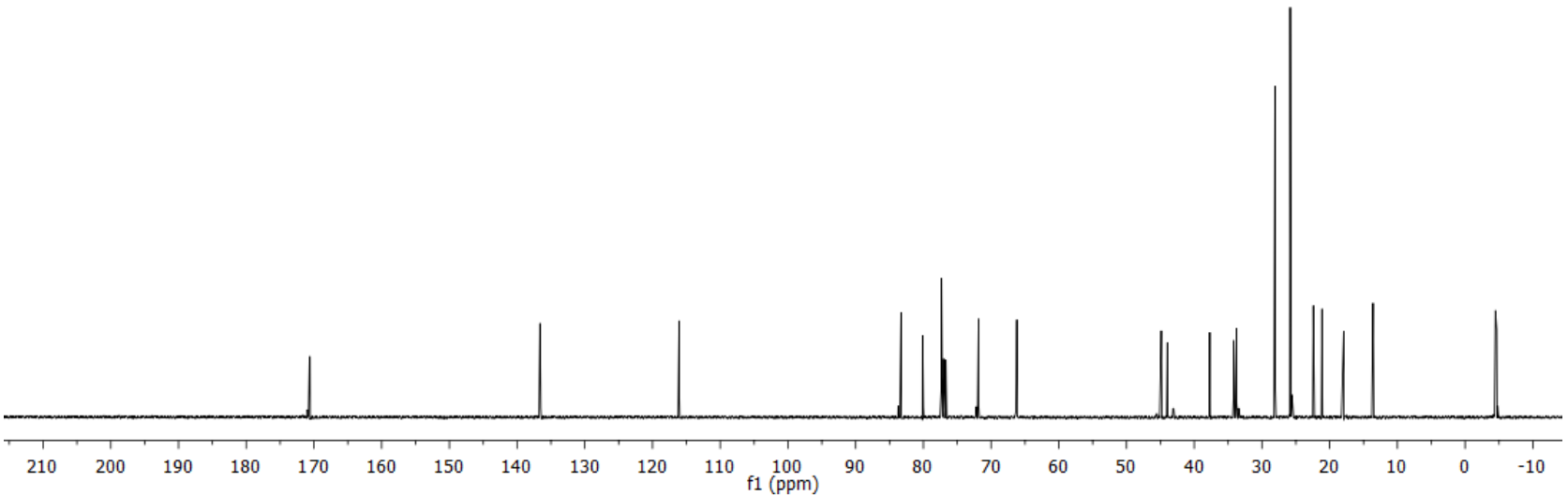
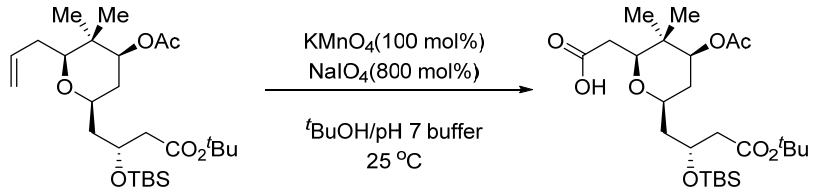
0.0
f1 (ppm)


Me
Me
OAc
O
CO2t-Bu
OTBS

9


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



9 **Fragment B**
81% Yield

A round bottom flask equipped with a magnetic stir bar was charged with $NaIO_4$ (529 mg, 2.47 mmol, 800 mol%) and $KMnO_4$ (48.9 mg, 0.309 mmol, 100 mol%). The solids were dissolved in 28.2 mL of pH 7 buffer (purchased from Fisher Chemical, SB107-500). The solution was stirred at room temperature under an atmosphere of argon for 20 min. The resulting dark purple solution was added via pipette to a solution of alkene **9** (149.8 mg, 0.309 mmol, 100 mol%) dissolved in *t*-BuOH (28.2 mL). The solution was stirred under an argon atmosphere for three hours then quenched by the addition of a saturated aqueous solution of $Na_2S_2O_3$. The layers were separated and the aqueous phase extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. A second round of drying was performed by diluting with methylene chloride, adding $MgSO_4$, filtering, and concentrating. The crude material was purified via flash column chromatography (10% followed by 15% ethyl acetate/hexanes with 0.5% acetic acid). Residual acetic acid was removed from the purified material by azeotroping with toluene three times to yield carboxylic acid **Fragment B** as a colorless oil (125.6 mg, 81%).

Characterization data for carboxylic acid **Fragment B**

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70 (dd, *J* = 11.6, 4.7 Hz, 1H), 4.17 (dtd, *J* = 7.7, 6.1, 3.7 Hz, 1H), 3.67 – 3.59 (m, 1H), 3.57 (dd, *J* = 9.6, 3.1 Hz, 1H), 2.51 (dd, *J* = 15.3, 3.1 Hz, 1H), 2.44 (d, *J* = 9.6 Hz, 1H), 2.38 (dd, *J* = 6.1, 2.3 Hz, 2H), 2.07 (s, 3H), 1.81 – 1.67 (m, 1H), 1.63 – 1.50 (m, 1H), 1.47 –1.42 (m, 1H), 1.43 (s, 9H), 0.92 (s, 3H), 0.88 – 0.84 (m, 1H), 0.86 (s, 9H), 0.85 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H).

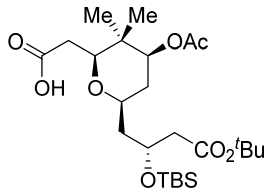
^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 170.8, 170.7, 80.4, 79.8, 76.8, 72.4, 66.3, 44.7, 43.8, 37.4, 35.3, 34.1, 28.1, 25.9, 22.3, 21.2, 17.9, 13.6, -4.7.

FTIR (neat) υ 2959, 2931, 2857, 1717, 1368, 1248, 1158, 1130, 836, 754 cm^{-1}.

HRMS (ESI) Calcd. for $C_{25}H_{46}O_8SiNa$ $[M+Na]^+$: 525.2860, Found: 525.2860.

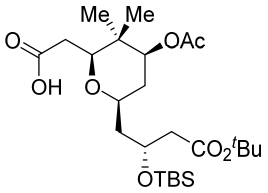
$[\alpha]^{22.5}_D$: +4.84° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.26 (30% ethyl acetate in hexanes).


Me
Me
OAc
O
OH
O
CO2tBu
OTBS

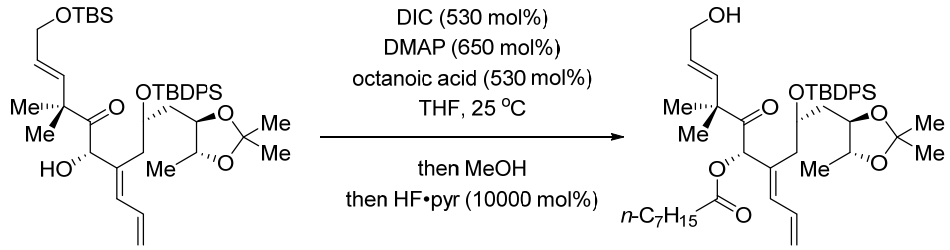
Fragment B

10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1
f1 (ppm)


Me
Me
OAc
O
OH
O
CO2tBu
OTBS

Fragment B

230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



2a **Fragment A**
84% Yield

A polyethylene vial was charged with alcohol **2a**[2] (18.6 mg, 0.026 mmol, 100 mol%) and was dissolved in freshly distilled THF (0.6 mL). DMAP (21 mg, 0.17 mmol, 650 mol%) was added, followed by octanoic acid (22 µL, 0.14 mmol, 530 mol%) and DIC (22 µL, 0.14 mmol, 530 mol%). The reaction was stirred at room temperature for 70 minutes at which point the starting alcohol **2a** had been consumed (determined by TLC). The reaction was cooled to 0 °C and methanol (0.5 mL) was added. The cooling bath was removed and the reaction was allowed to warm to room temperature and stir for 1.5 hours. The reaction was cooled back down to 0 °C and HF•pyridine (72 µL of a solution that was 70% HF, 2.74 mmol, 10,000 mol%) was added dropwise over five minutes. The cooling bath was removed and the reaction was allowed to stir a room temperature for 1 hour. The reaction was then quenched by the careful addition of $NaHCO_{3(aq)}$ (Caution, gas evolution!!). The reaction was then poured into brine and extracted three times with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered and concentrated. The crude residue was then purified by flash column chromatography (1→3% EtOAc in DCM) to provide allylic alcohol **Fragment A** (16.5 mg, 84%).

Characterization data for allylic alcohol **Fragment A**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (m, 7.76 – 7.69 4H), 7.51 – 7.32 (m, 6H), 6.34 (ddd, *J* = 16.7, 11.0, 10.2 Hz, 1H), 5.92 (d, *J* = 11.0 Hz, 1H), 5.52 – 5.48 (m, 2H), 5.24 (dd, *J* = 16.7, 1.5 Hz, 1H), 5.18 (dd, *J* = 10.1, 1.5 Hz, 1H), 4.15 – 4.04 (m, 1H), 3.88 – 3.73 (m, 3H), 3.51 (dq, *J* = 8.5, 5.9 Hz, 1H), 2.46 (dd, *J* = 13.3, 9.6 Hz, 1H), 2.34 – 2.07 (m, 3H), 1.70 – 1.39 (m, 4H), 1.33 – 1.23 (m, 16H), 1.19 – 1.14 (m, 6H), 1.07 (s, 3H), 1.04 (s, 9H), 0.88 (t, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 172.7, 136.1, 135.9, 135.8, 135.0, 134.4, 133.4, 131.9, 130.0, 129.9, 129.6, 128.8, 127.7, 127.5, 121.3, 107.7, 78.7, 78.4, 76.8, 70.0, 63.0, 48.8, 39.7, 38.6, 33.5, 31.6, 29.0, 28.9, 27.4, 27.2, 26.9, 24.5, 24.5, 24.3, 22.5, 19.4, 16.9, 14.0.

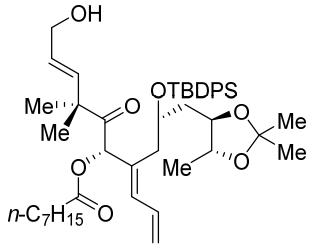
FTIR (neat) υ 3444, 2954, 2931, 2857, 1740, 1718, 1462, 1427, 1378, 1165, 1087, 1059, 923, 739 cm^{-1}.

$[\alpha]^{23.0}_D$: +57.88° (c = 1.3, $CHCl_3$).

HRMS (ESI) Calcd. for $C_{45}H_{66}O_7SiNa$ $[M+Na]^+$: 770.4501, Found: 770.4495.

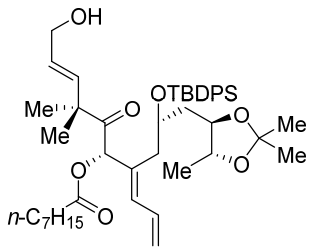
TLC (SiO_2): R_f = 0.45 (30% ethyl acetate in hexanes).

[2] Lu, Y.; Woo, S. K.; Krische, M. J. *J. Am. Chem. Soc.* **2011**, *133*, 13876.


OH
OTBDPS
Me
Me
O
O
Me
Me
Me
O
O
n-C7H15
O

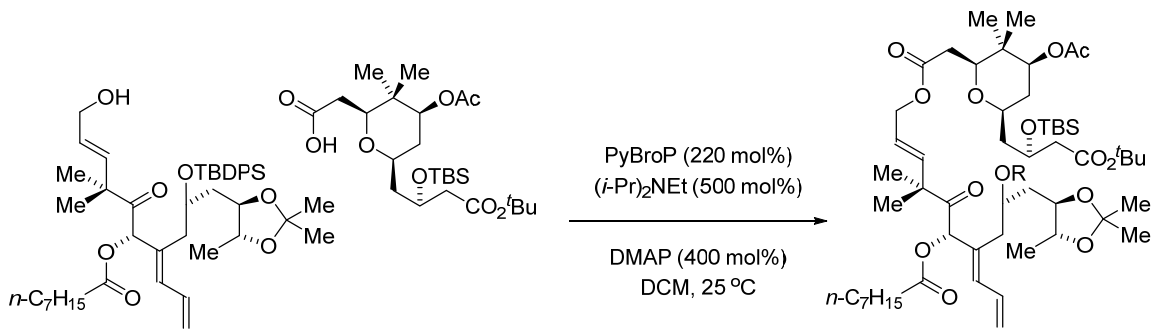
Fragment A

10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1
f1 (ppm)


OH
OTBDPS
Me
Me
O
O
Me
Me
Me
O
O
n-C7H15
O

Fragment A

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)



A round bottom flask was charged with allylic alcohol **Fragment A** (171 mg, 0.23 mmol, 100 mol%) and carboxylic acid **Fragment B** (150 mg, 0.30 mmol, 130 mol%) and dissolved in freshly distilled dichloromethane (135 mL). Hunig's base (192 μL, 1.15 mmol, 500 mol%) was added via syringe. DMAP (112 mg, 0.92 mmol, 400 mol%) and PyBrOP (235 mg, 0.50 mmol, 220 mol%) were added simultaneously as solids in a single portion. The reaction was stirred at room temperature for 2 hours at room temperature. The crude reaction mixture was loaded directly onto a column of silica gel and purified by flash column chromatography (10% then 20 % Et_2O/Hexanes) to yield the ester **10** (271 mg, 96%) as a colorless oil.

Characterization data for ester **10**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 – 7.68 (m, 4H), 7.46 – 7.34 (m, 6H), 6.31 (dt, *J* = 16.8, 10.4 Hz, 1H), 5.93 (d, *J* = 10.9 Hz, 1H), 5.64 (dt, *J* = 15.7, 1.2 Hz, 1H), 5.44 (dt, *J* = 15.8, 6.1 Hz, 1H), 5.23 (dd, *J* = 16.8, 1.2 Hz, 1H), 5.16 – 5.13 (m, 2H), 4.69 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.39 (ddd, *J* = 12.8, 6.4, 1.2 Hz, 1H), 4.32 (ddd, *J* = 12.9, 5.8, 1.3 Hz, 1H), 4.20 – 4.08 (m, 2H), 3.78 – 3.71 (m, 1H), 3.60 (dd, *J* = 8.9, 3.3 Hz, 1H), 3.54 (tq, *J* = 9.0, 1.6 Hz, 1H), 3.51 –3.45 (m, 1H), 2.46 – 2.31 (m, 5H), 2.29 – 2.22 (m, 1H), 2.21 – 2.14 (m, 1H), 2.12 (dd, *J* = 13.4, 4.6 Hz, 1H), 2.05 (s, 3H), 1.75 (ddd, *J* = 12.5, 4.7, 2.3 Hz, 1H), 1.63 – 1.51 (m, 6H), 1.42 (s, 9H), 1.31 – 1.23 (m, 15H), 1.17 – 1.13 (m, 6H), 1.09 (s, 2H), 1.06 – 1.03 (m, 10H), 0.91 – 0.84 (m, 15H), 0.84 – 0.81 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H).

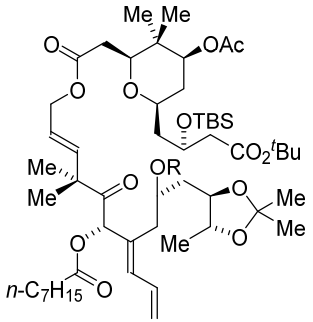
^{13}C NMR (150 MHz, $CDCl_3$) δ 206.4, 172.7, 171.1, 170.6, 170.5, 138.2, 136.4, 135.9, 135.8, 134.2, 133.8, 131.9, 129.8, 129.8, 129.7, 127.7, 127.6, 123.5, 121.3, 107.7, 80.1, 79.8, 78.9, 78.4, 76.9, 76.8, 49.0, 44.5, 43.8, 39.3, 38.8, 37.4, 35.4, 34.1, 33.5, 31.6, 29.0, 28.9, 28.2, 28.1, 28.1, 27.4, 27.2, 26.9, 25.8, 25.8, 25.8, 24.6, 24.5, 24.4, 22.6, 22.3, 21.1, 19.4, 17.9, 16.9, 14.0, 13.6, -4.6, -4.7.

FTIR (neat) υ 2954, 2930, 2857, 1740, 1472, 1367, 1243, 1163, 1086, 1006, 837, 704 cm^{-1}.

HRMS (ESI) Calcd. for $C_{70}H_{110}O_{14}Si_2Na$ $[M+Na]^+$: 1253.7326, Found: 1253.7301.

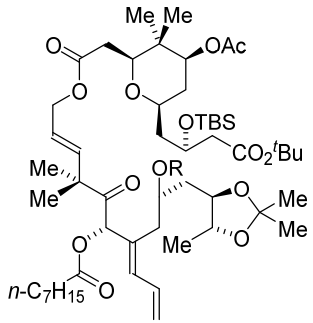
$[\alpha]^{22.8}_D$: +28.33° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.10 (10% ethyl acetate in hexanes).


Me Me
OAc
O
O
O
OTBS
CO2tBu
Me
Me
OR
O
O
Me
Me
O
Me
O
n-C7H15
O

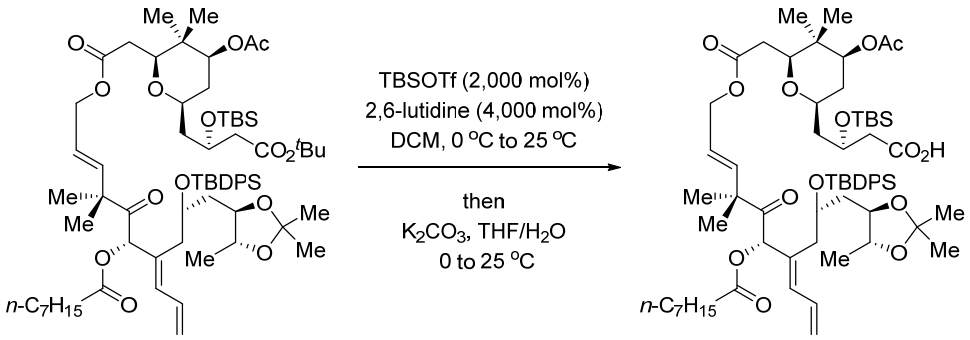
10, R = TBDPS

8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0
f1 (ppm)


Me Me
OAc
O
O
O
OTBS
CO2tBu
Me
Me
OR
O
O
Me
Me
Me
O
O
n-C7H15
O

10, R = TBDPS

230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



10 **10a** 96% yield

A round bottom flask equipped with a magnetic stirring bar was charged with *tert*-butyl ester **10** (271 mg, 0.22 mmol, 100 mol%) and put under an atmosphere of argon. The residue was dissolved in dichloromethane (8.6 mL) and the solution cooled to 0 °C. 2,6-lutidine (1.02 mL, 8.8 mmol, 4,000 mol%) was added followed by dropwise addition of TBS triflate (1.01 mL, 4.4 mmol, 2,000 mol%). The reaction was stirred for five minutes at 0 °C then allowed to warm to room temperature and stir overnight. The reaction was then cooled to 0 °C and K_2CO_3 (76 mg, 0.55 mmol, 250 mol%) in THF/H_2O was added. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was cooled to 0 °C, diluted with water, and acidified with $NaHSO_{4(aq)}$. The layers were separated and the aqueous phase extracted three times with ethyl acetate. The combined organic phases were washed with water, washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified via flash column chromatography (20% → 40% Et_2O/Hex) to yield carboxylic acid **10a** (248 mg, 96%) as a white foam.

Characterization data for carboxylic acid **10a**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.63 (m, 4H), 7.48 – 7.32 (m, 6H), 6.31 (dt, *J* = 16.7, 10.5 Hz, 1H), 5.93 (d, *J* = 10.9 Hz, 1H), 5.62 (dt, *J* = 15.7, 1.3 Hz, 1H), 5.42 (dt, *J* = 15.8, 6.0 Hz, 1H), 5.22 (dd, *J* = 16.5, 1.8 Hz, 1H), 5.17 – 5.11 (m, 2H), 4.68 (dd, *J* = 11.6, 4.7 Hz, 1H), 4.35 (dd, 6.2, 1.3 Hz, 1H), 4.31 (dd, 6.2, 1.3 Hz, 1H), 4.22 –4.15 (m, 1H), 4.14 – 4.06 (m, 1H), 3.74 (ddd, *J* = 10.0, 8.5, 1.9 Hz, 1H), 3.64 – 3.40 (m, 2H), 2.52 (dd, *J* = 15.0, 4.9 Hz, 1H), 2.45 – 2.33 (m, 4H), 2.30 – 2.08 (m, 3H), 2.04 (s, 3H), 1.81 – 1.64 (m, 2H), 1.64 – 1.49 (m, 3H), 1.48 – 1.36 (m, 3H), 1.33 – 1.20 (m, 17H), 1.19 – 1.10 (m, 7H), 1.05 (d, *J* = 16.5 Hz, 14H), 0.95 – 0.78 (m, 20H), 0.06 (s, 3H), 0.04 (s, 3H).

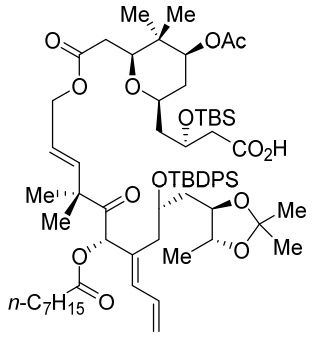
^{13}C NMR (125 MHz, $CDCl_3$) δ 206.3, 175.5, 172.8, 171.1, 170.5, 138.0, 136.4, 135.8, 135.8, 134.1, 133.7, 131.9, 129.7, 129.7, 129.6, 127.6, 127.5, 123.4, 121.2, 107.6, 79.9, 78.9, 78.4, 76.8, 76.7, 72.4, 69.9, 66.7, 64.7, 48.9, 43.7, 42.9, 39.3, 38.8, 37.3, 35.2, 34.1, 33.5, 31.6, 28.9, 28.9, 27.4, 27.1, 26.9, 25.7, 24.5, 24.4, 24.3, 22.5, 22.2, 21.0, 19.3, 17.8, 16.9, 14.0, 13.6, -4.7, -4.9.

FTIR (neat) υ 2954, 2930, 2857, 1740, 1720, 1471, 1428, 1378, 1242, 1166, 1111, 1085, 703 cm^{-1}.

HRMS (ESI) Calcd. for $C_{66}H_{102}O_{14}Si_2Na$ $[M+Na]^+$: 1197.6706, Found: 1197.6273.

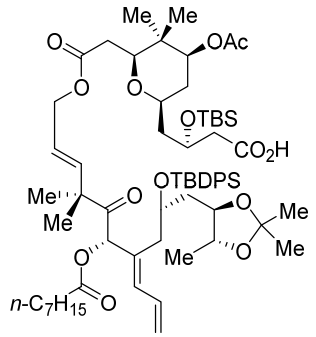
$[\alpha]^{30.5}_D$: +44.66° (c = 1.0 , $CHCl_3$).

TLC (SiO_2): R_f = 0.43 (30% ethyl acetate in hexanes).


Me
Me
OAc
O
O
O
OTBS
CO2H
OTBDPS
Me
O
Me
Me
O
Me
O
Me
n-C7H15
O

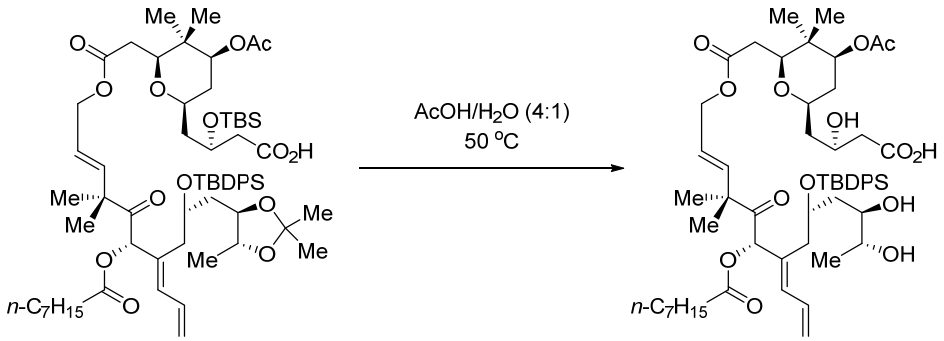
10a

8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Me
Me
OAc
O
O
O
OTBS
CO2H
OTBDPS
Me
O
O
Me
Me
Me
Me
O
n-C7H15
O

10a

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



10a **10b**
84% yield

A round bottom flask was charged with acetonide **10a** (174 mg, 0.15 mmol) and 3.75 mL of a 4:1 mixture of acetic acid and water was added. The reaction was capped with a septum and an argon balloon and stirred at 50 °C for 9 hours and ten minutes. The reaction was cooled to room temperature and diluted with toluene. The reaction was concentrated and azeotroped two additional times with fresh toluene. The crude residue was purified via flash column chromatography (0 → 1% MeOH/DCM with 0.5% AcOH) to yield triol-acid **10b** (127 mg, 84%) as a white foam.

Characterization data for triol-acid acid **10b**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.66 (m, 4H), 7.59 – 7.35 (m, 6H), 6.37 (dt, *J* = 16.7, 10.5 Hz, 1H), 5.92 (d, *J* = 10.9 Hz, 1H), 5.61 (d, *J* = 15.7 Hz, 1H), 5.48 (dt, *J* = 15.8, 6.2 Hz, 1H), 5.24 (d, *J* = 17.4 Hz, 1H), 5.20 (d, *J* = 10.8 Hz, 1H), 5.11 (s, 1H), 4.71 (dd, *J* = 11.5, 4.7 Hz, 1H), 4.44 (qd, *J* = 12.8, 6.1 Hz, 2H), 4.26 – 4.10 (m, 3H), 3.77 (d, *J* = 10.6 Hz, 1H), 3.71 – 3.64 (m, 1H), 3.59 (dd, *J* = 9.6, 3.8 Hz, 1H), 3.90 – 3.40 (m, 1H), 2.71 (dd, *J* = 13.6, 9.7 Hz, 1H), 2.53 – 2.40 (m, 4H), 2.28 – 2.08 (m, 3H), 2.06 (s, 4H), 1.84 – 1.75 (m, 1H), 1.65 (ddd, *J* = 20.1, 10.6, 5.3 Hz, 2H), 1.56 – 1.43 (m, 5H), 1.36 – 1.20 (m, 10H), 1.17 (s, 3H), 1.10 (s, 3H), 1.08 – 1.02 (m, 12H), 0.94 (s, 3H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.85 (s, 3H).

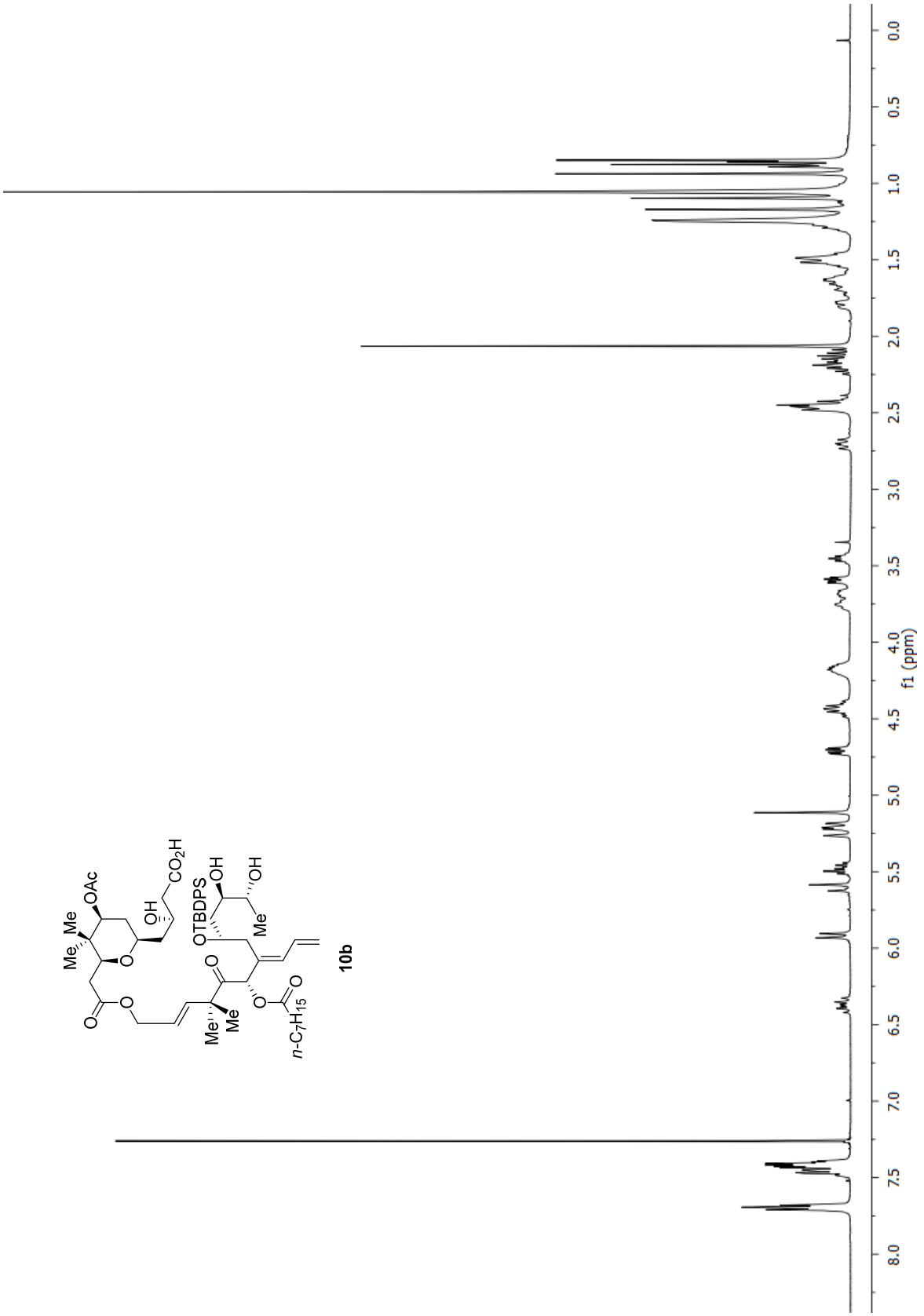
^{13}C NMR (151 MHz, $CDCl_3$) δ 206.4, 174.9, 172.8, 171.6, 170.5, 138.4, 136.5, 135.9, 135.7, 133.2, 132.9, 131.7, 130.1, 130.1, 129.5, 127.9, 127.9, 123.6, 121.8, 80.3, 78.6, 76.7, 72.9, 72.8, 71.0, 71.0, 65.2, 65.1, 49.0, 41.1, 40.8, 37.5, 37.4, 37.3, 34.8, 33.7, 33.5, 31.6, 29.6, 28.9, 28.8, 26.9, 26.9, 24.6, 24.4, 24.3, 22.5, 22.2, 21.0, 19.1, 19.0, 14.0, 13.7.

FTIR (neat) υ 3479, 2954, 2930, 2857, 1737, 1720, 1428, 1365, 1310, 1244, 1162, 1110, 1085, 979, 742, 705 cm^{-1}.

HRMS (ESI) Calcd. for $C_{57}H_{84}O_{14}SiNa$ $[M+Na]^+$: 1043.5528, Found: 1043.5521.

$[\alpha]^{21.6}_D$: +34.50° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.13 (3% methanol in dichloromethane with 0.1% AcOH).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Me
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OH
CO2H
O
O
OTBDPS
OH
Me
OH
O
Me
Me
O
O
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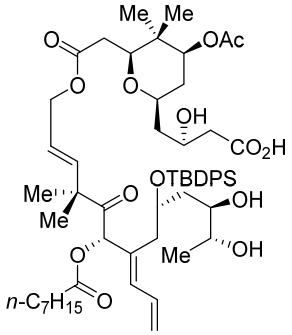
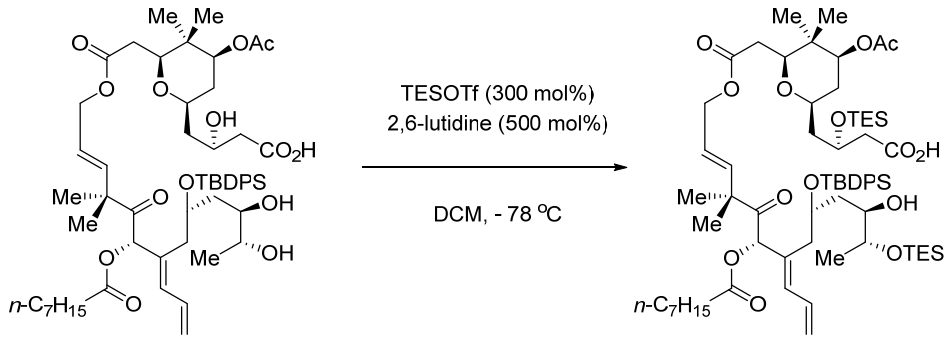
10b
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Me
Me
OAc
O
O
O
OH
CO2H
OTBDPS
Me
Me
O
OH
O
Me
OH
n-C7H15
O

10b

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



10b **11**
63% yield

A round bottom flask equipped with a magnetic stir bar was charged with triol-acid **10b** (79.6 mg, 0.0779 mmol, 100 mol%) and put under an argon atmosphere. The residue was dissolved in dichloromethane (3.9 mL) and 2,6-lutidine (45.1 μL, 0.390 mmol, 500 mol%) was added. The mixture was cooled to -78 °C and TES-triflate (54 μL, 0.234 mmol, 300 mol%) was added dropwise over the course of fifteen minutes. The reaction was stirred for an additional fifty minutes at -78 °C then quenched by the addition of pH 4 buffer while still at this temperature. The cooling bath was removed and the reaction warmed to room temperature and stirred for three hours and ten minutes. The crude mixture was poured into brine and the layers separated. The aqueous phase was extracted three times with dichloromethane. The combined organic extracts were washed with a mixture of ice and 1M $HCl_{(aq)}$, then with water, and finally with brine. The solution was dried over Na_2SO_4, filtered and concentrated. The crude residue was purified via flash column chromatography (20% EtOAc/Hex) to yield hydroxyl-acid **11** (60.9 mg, 63%) as a yellow oil.

Characterization data for hydroxy-acid **11**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72 (ddt, *J* = 6.3, 4.7, 1.4 Hz, 4H), 7.46 – 7.36 (m, 6H), 6.37 (dt, *J* = 16.8, 10.5 Hz, 1H), 5.88 (d, *J* = 10.7 Hz, 1H), 5.60 (dt, *J* = 15.8, 1.3 Hz, 1H), 5.43 (dt, *J* = 15.7, 6.1 Hz, 1H), 5.22 (dd, *J* = 16.8, 1.0 Hz, 1H), 5.16 (dd, *J* = 10.2, 2.0 Hz, 1H), 5.00 (s, 1H), 4.68 (dd, *J* = 11.6, 4.8 Hz, 1H), 4.39 (ddd, *J* = 12.8, 6.4, 1.3 Hz, 1H), 4.35 (ddd, *J* = 12.8, 5.9, 1.4 Hz, 1H), 4.24 – 4.16 (m, 1H), 3.65 – 3.58 (m, 2H), 3.57 – 3.50 (m, 2H), 2.62 – 2.58 (m, 1H), 2.57 (dd, *J* = 10.5, 5.0 Hz, 1H), 2.50 (dd, *J* = 15.6, 5.3 Hz, 1H), 2.45 – 2.36 (m, 2H), 2.28 – 2.15 (m, 2H), 2.10 – 2.07 (dd, *J* = 13.5, 4.5 Hz, 1H), 2.06 (s, 3H), 1.78 – 1.71 (m, 2H), 1.63 – 1.57 (m, 2H), 1.57 – 1.50 (m, 2H), 1.48 – 1.37 (m, 2H), 1.32 – 1.20 (m, 11H), 1.11 (s, 3H), 1.08 (d, *J* = 6.2 Hz, 3H), 1.06 (s, 3H), 1.04 (s, 9H), 0.96 (t, *J* = 7.9 Hz, 9H), 0.93 (t, *J* = 8.0 Hz, 9H), 0.91 (s, 3H), 0.88 (t, *J* = 7.0 Hz, 3H), 0.83 (s, 3H), 0.63 (qd, *J* = 7.9, 1.2 Hz, 6H), 0.57 (qd, *J* = 7.9, 2.3 Hz, 6H).

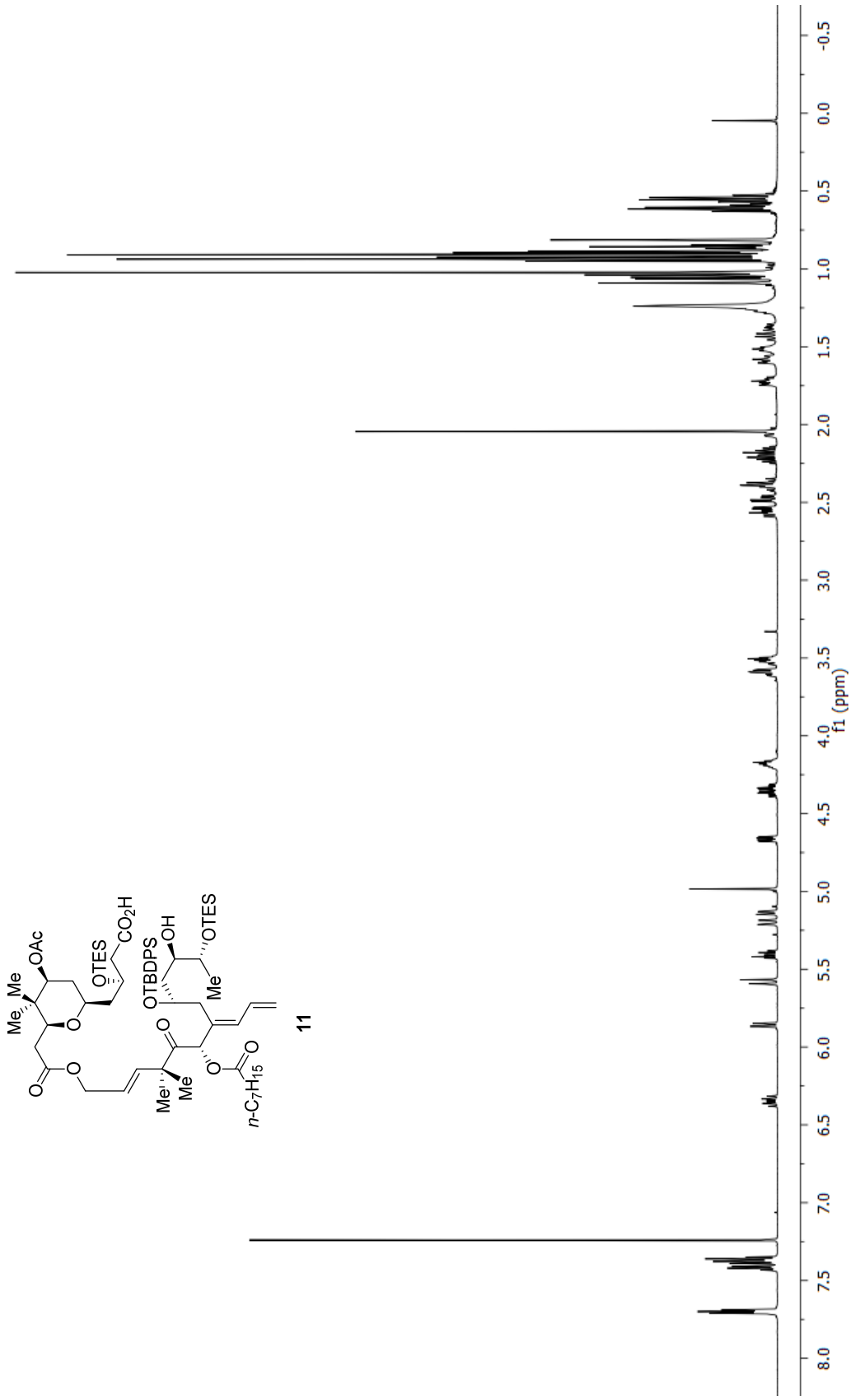
^{13}C NMR (150 MHz, $CDCl_3$) δ 206.3, 172.9, 172.4, 171.4, 170.6, 138.2, 135.9, 135.9 (two carbons), 134.2, 133.6, 131.9, 129.9, 129.9, 129.7, 127.8, 127.7, 123.4, 121.1, 80.1, 78.3, 72.7, 72.1, 72.0, 70.0, 67.3, 64.9, 48.9, 43.6, 42.3, 39.2, 37.3, 35.1, 34.0, 33.7, 31.7, 29.7, 29.1, 28.9, 26.9, 24.7, 24.5, 24.2, 22.6, 22.3, 21.1, 20.0, 19.4, 14.1, 13.7, 6.8, 6.8, 5.0, 4.7.

FTIR (neat) υ 2955, 2932, 2875, 2856, 1741, 1719, 1459, 1427, 1376, 1305, 1240, 1164, 1075, 1005, 740, cm^{-1}.

HRMS (ESI) Calcd. for $C_{69}H_{112}O_{14}Si_3Na$ $[M+Na]^+$: 1271.7258, Found: 1271.7283 .

$[\alpha]^{30.5}_D$: +30.61° (c = 0.5, $CHCl_3$).

TLC (SiO_2): R_f = 0.21 (20% ethyl acetate in hexanes).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Me
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OTES
CO2H
OTBDPS
OH
OTES
Me
Me
Me
n-C7H15
11
8.0
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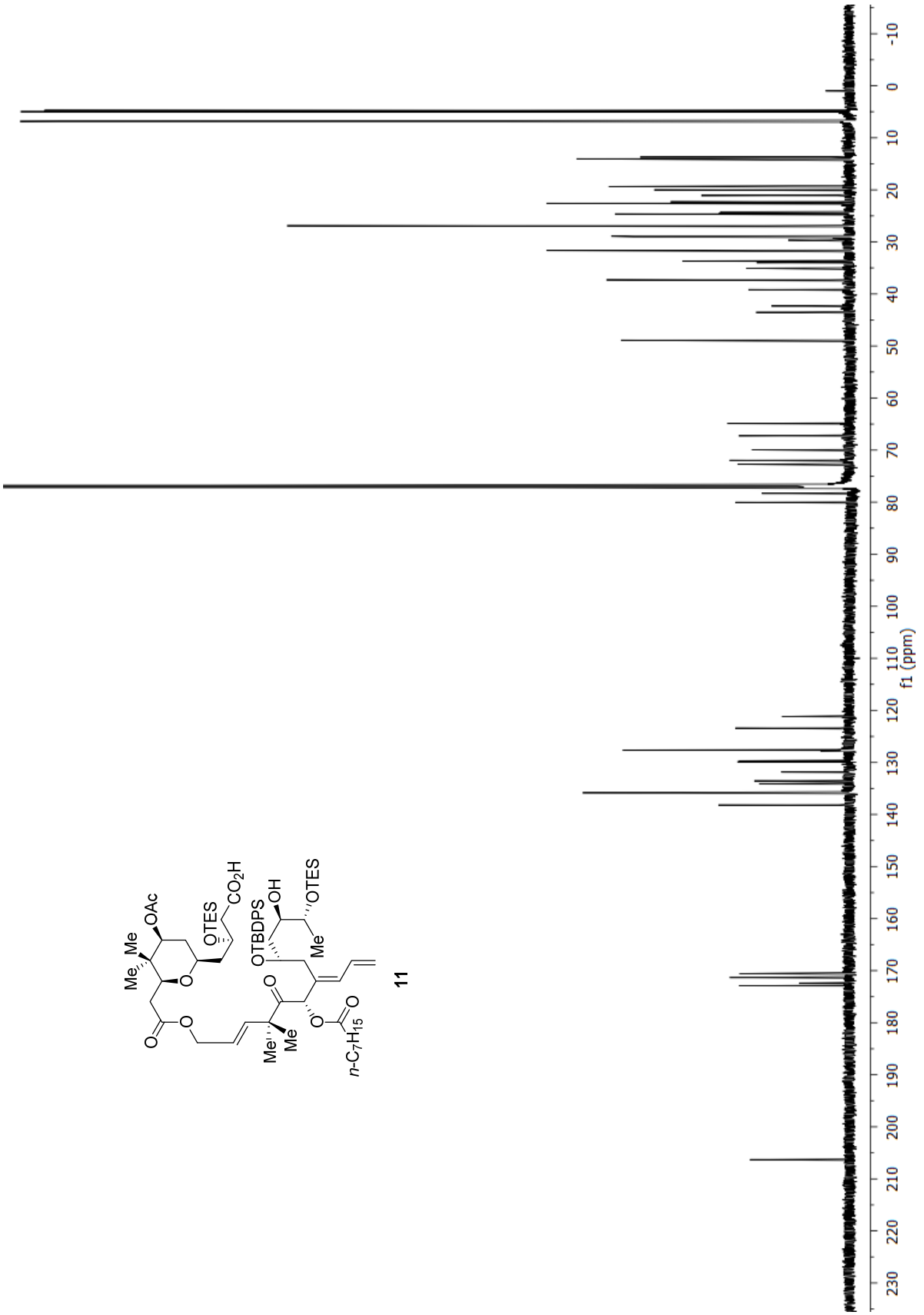
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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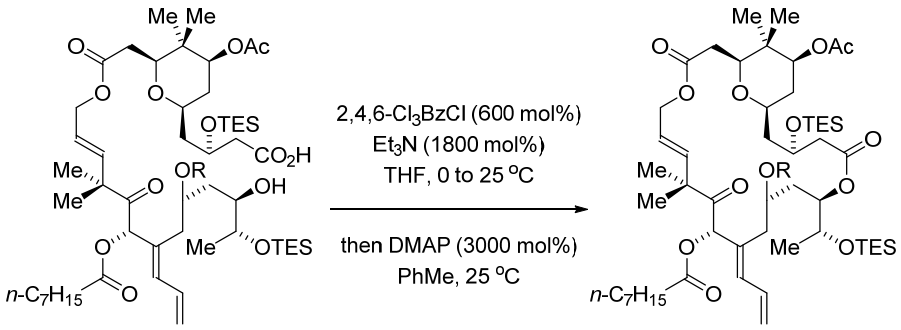
Me
OAc
OTES
CO2H
OTBDPS
OH
OTES
Me
Me
Me
n-C7H15
11
f1 (ppm)



11, R = TBDPS

12, R = TBDPS
85% yield

A glass vial was charged with hydroxyl-acid **11** (45 mg, 0.036 mmol, 100 mol%) and placed under an argon atmosphere. THF (9 mL) was added and the solution was cooled to 0 °C. Triethylamine (91 μL, 0.65 mmol, 1800 mol%) was added followed by 2,4,6-trichlorobenzoyl chloride (34 μL, 0.22 mmol, 600 mol%). The ice bath was removed and the reaction was stirred at room temperature for three hours and twenty minutes. The reaction was diluted with toluene (25 mL) and slowly added via syringe pump to a solution of DMAP (132 mg, 1.08 mmol, 3000 mol%) in toluene (38 mL) over twelve hours. The reaction was stirred for an additional eight hours at room temperature then quenched by addition of saturated $NaHCO_{3(aq)}$. The layers were separated and the aqueous phase was extracted three additional times with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. The crude reaction mixture was purified via flash column chromatography (5% → 10% EtOAc/Hex) to yield macrolactone **12** (37.8 mg, 85%) as a thin film.

Characterization data for macrolactone **12**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.64 (m, 4H), 7.50 – 7.30 (m, 6H), 6.08 – 5.94 (m, 1H), 5.89 (d, *J* = 10.9 Hz, 1H), 5.81 (dt, *J* = 15.6, 1.3 Hz, 1H), 5.75 (s, 1H), 5.57 (ddd, *J* = 15.7, 6.7, 5.8 Hz, 1H), 5.13 (dd, *J* = 16.4, 1.9 Hz, 1H), 5.01 (dd, *J* = 9.8, 2.0 Hz, 1H), 4.90 (dt, *J* = 8.6, 4.4 Hz, 1H), 4.68 (dd, *J* = 11.6, 4.7 Hz, 1H), 4.54 (ddd, *J* = 12.8, 5.7, 1.3 Hz, 1H), 4.44 (ddd, *J* = 12.8, 6.7, 1.2 Hz, 1H), 4.19 – 4.02 (m, 2H), 3.87 – 3.75 (m, 1H), 3.65 – 3.53 (m, 1H), 3.48 (dd, *J* = 11.3, 8.9 Hz, 1H), 2.51 – 2.37 (m, 3H), 2.38 – 2.12 (m, 4H), 2.07 (s, 3H), 1.86 (ddd, *J* = 14.0, 7.5, 4.3 Hz, 1H), 1.77 (ddd, *J* = 12.5, 4.8, 2.3 Hz, 1H), 1.72 – 1.51 (m, 5H), 1.48 – 1.43 (m, 1H), 1.40 (s, 3H), 1.36 – 1.22 (m, 12H), 1.16 (s, 3H), 1.02 –0.98 (m, 11H), 0.96 – 0.89 (m, 18H), 0.89 – 0.84 (m, 4H), 0.80 (d, *J* = 6.3 Hz, 3H), 0.64 – 0.49 (m, 12H).

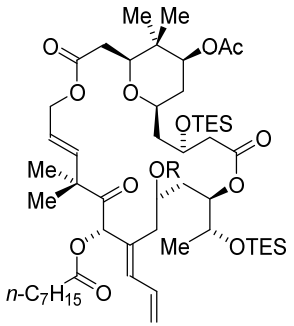
^{13}C NMR (126 MHz, $CDCl_3$) δ 207.0, 173.0, 171.5, 171.0, 170.4, 138.5, 136.1, 136.0, 135.8, 134.3, 133.8, 132.2, 130.8, 129.7, 129.5, 127.7, 127.4, 124.1, 120.4, 80.4, 78.5, 76.8, 73.6, 73.0, 70.6, 67.9, 67.6, 64.8, 49.6, 44.0, 43.4, 37.2, 37.1, 36.5, 35.2, 34.3, 33.8, 31.7, 29.1, 28.9, 27.0, 24.7, 24.7, 24.2, 22.6, 22.3, 21.1, 19.3, 17.7, 14.1, 13.7, 6.9, 6.8, 4.9, 4.9.

FTIR (neat) υ 3066, 3039, 2955, 2933, 2875, 1740, 1461, 1427, 1378, 1308, 1241, 1163, 1110 cm^{-1}.

HRMS (ESI) Calcd. for $C_{69}H_{110}O_{13}Si_3Na$ $[M+Na]^+$: 1253.7152, Found: 1253.7173.

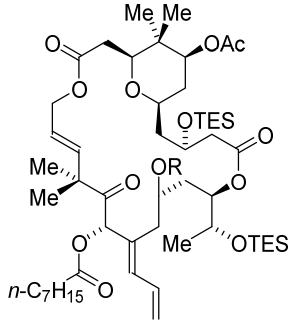
$[\alpha]^{28.2}_D$: +16.0° (c = 1.0, $CHCl_3$).

TLC (SiO_2): R_f = 0.19 (10% ethyl acetate in hexanes).


Me Me
OAc
O
O
O
OTES
O
Me
OR
Me
O
O
Me
OTES
O
n-C7H15
O

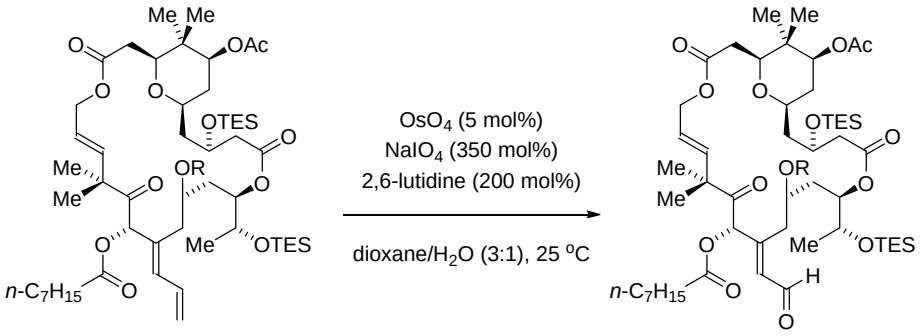
12, R = TBDPS

8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Me Me
OAc
O
O
O
OTES
O
Me
Me
OR
O
O
Me
OTES
O
n-C7H15
O

12, R = TBDPS

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



12 **12a**, R = TBDPS
64% yield

A glass vial equipped with a magnetic stir bar was charged with diene **12** (39 mg, 0.032 mmol, 100 mol%) and placed under an argon atmosphere. The starting was material was dissolved in dioxane (1.26 mL) and water (423 μL) was added (the solution became cloudy upon addition of water due to incomplete solubility). To this vial was added 2,6-lutidine (7.3 μL, 0.063 mmol, 200 mol%) followed by OsO_4 (0.4 mg, 0.0016 mmol, 5 mol%, added by addition of 403 μL of a 1 mg/mL solution of OsO_4 in THF). $NaIO_4$ (23.7 mg, 0.11 mmol, 350 mol%) was added as a solid in a single portion. The reaction was stirred at room temperature for 22.5 hours during which time a significant amount of white precipitate was formed. The reaction was diluted with pH 7 buffer and poured into brine. The layers were separated and the aqueous phase was extracted three times with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified via flash column chromatography (5 → 10% EtOAc/Hex) to yield aldehyde **12a** (25 mg, 64%) as a thin film.

Characterization data for aldehyde **12a**

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (d, *J* = 7.7 Hz, 1H), 7.75 – 7.59 (m, 4H), 7.50 – 7.32 (m, 6H), 6.07 (d, *J* = 7.7 Hz, 1H), 5.80 (d, *J* = 0.8 Hz, 1H), 5.66 (d, *J* = 15.8 Hz, 1H), 5.58 (dt, *J* = 15.8, 5.9 Hz, 1H), 4.74 – 4.64 (m, 3H), 4.52 (qd, *J* = 13.1, 5.9 Hz, 2H), 4.25 – 4.11 (m, 2H), 3.70 – 3.61 (m, 1H), 3.56 – 3.48 (m, 1H), 2.74 (dd, *J* = 13.9, 8.4 Hz, 1H), 2.62 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.51 – 2.31 (m, 4H), 2.29 – 2.10 (m, 2H), 2.09 (s, 3H), 1.92 (dt, *J* = 14.3, 5.2 Hz, 1H), 1.78 (ddd, *J* = 12.4, 4.7, 2.3 Hz, 1H), 1.73 – 1.64 (m, 1H), 1.55 – 1.43 (m, 5H), 1.38 – 1.20 (m, 12H), 1.15 (s, 3H), 0.98 – 0.96 (m, 12H), 0.96 – 0.95 (m, 2H), 0.94 – 0.93 (m, 4H), 0.92 – 0.91 (m, 4H), 0.91 – 0.90 (m, 1H), 0.90 – 0.89 (m, 4H), 0.89 – 0.88 (m, 2H), 0.87 (s, 3H), 0.87 (s, 3H), 0.73 (d, *J* = 6.3 Hz, 3H), 0.66 – 0.43 (m, 12H).

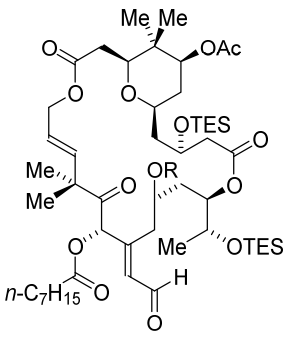
^{13}C NMR (151 MHz, $CDCl_3$) δ 204.6, 191.2, 172.1, 171.5, 170.6, 152.5, 137.9, 136.0, 136.0, 135.8, 133.9, 133.5, 129.9, 129.8, 127.7, 127.6, 125.3, 80.4, 77.8, 76.9, 73.7, 73.1, 70.8, 68.4, 67.8, 64.8, 50.0, 44.1, 43.3, 37.3, 36.5, 35.1, 34.3, 33.6, 31.7, 29.7, 29.0, 28.9, 26.9, 24.7, 24.6, 23.7, 22.6, 22.3, 21.1, 19.2, 17.9, 14.1, 14.1, 13.7, 6.9, 6.8, 4.9, 4.8.

FTIR (neat) υ 3052, 2954, 2933, 2876, 2847, 1742, 1682, 1463, 1377, 1242, 1109, 1084, 741, 704 cm^{-1}.

HRMS (ESI) Calcd. for $C_{68}H_{108}O_{14}Si_3Na$ $[M+Na]^+$: 1255.6954 , Found: 1255.6914 .

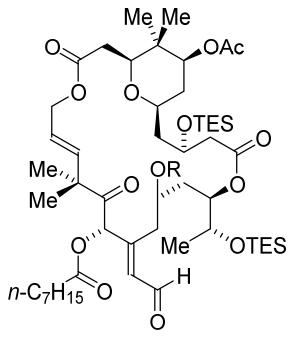
$[\alpha]^{29.4}_D$: +11.1° (c = 0.5, $CHCl_3$).

TLC (SiO_2): R_f = 0.44 (20% ethyl acetate in hexanes).


Me Me
OAc
O
O
O
OTES
O
Me
Me
O
OR
O
O
Me
OTES
n-C7H15
O
H
O

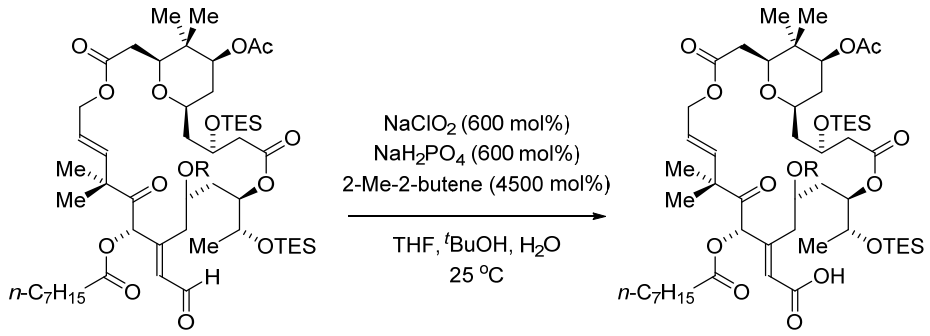
12a, R = TBDPS

10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Me Me
OAc
O
O
O
OTES
O
Me
Me
O
OR
O
O
Me
OTES
H
n-C7H15
O
O

12a, R = TBDPS

240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



12a, R = TBDPS

13, R = TBDPS
92% yield

Aldehyde **12a** (11.8 mg, 0.0096 mmol, 100 mol%) was dissolved in THF (1 mL) and ^{t}BuOH (1 mL). To this solution was added 2-methyl-2-butene (215 µL of a 2 M solution in THF, 0.43 mmol, 4500 mol%), then an aqueous solution comprised of $NaClO_2$ (5.2 mg, 0.057 mmol, 600 mol%) and NaH_2PO_4 (7.9 mg, 0.057 mmol, 600 mol%) in H_2O (250 µL) was added to the reaction. The reaction was stirred at room temperature for 2 hours and 45 minutes then diluted with pH 7 buffer and quenched with $Na_2S_2O_{3(aq)}$. The mixture was poured into brine and the layers were separated. The aqueous phase was extracted three times with ethyl acetate. The combined organics were dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified via flash column chromatography (5 → 15% EtOAc/Hexanes) to yield carboxylic acid **13** (11 mg, 92%) as a thin film.

Characterization data for carboxylic acid **13**

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 – 7.65 (m, 4H), 7.50 – 7.30 (m, 6H), 6.10 (s, 1H), 5.78 (s, 1H), 5.71 – 5.41 (m, 2H), 4.70 (dd, *J* = 11.6, 4.7 Hz, 1H), 4.70 – 4.63 (m, 1H), 4.54 (dd, *J* = 12.9, 5.6 Hz, 1H), 4.48 (dd, *J* = 13.0, 4.7 Hz, 1H), 4.28 (ddd, *J* = 13.5, 8.3, 5.7 Hz, 1H), 4.17 – 4.11 (m, 1H), 3.71 – 3.63 (m, 1H), 3.61 (dd, *J* = 10.5, 2.3 Hz, 1H) 3.56 – 3.50 (m, 1H), 2.85 (s, 1H), 2.60 – 2.49 (m, 2H), 2.50 – 2.32 (m, 3H), 2.26 – 2.15 (m, 1H), 2.12 – 2.02 (m, 1H), 2.07 (s, 3H), 1.97 (ddd, *J* = 14.5, 5.8, 3.7 Hz, 1H), 1.78 (ddd, *J* = 12.8, 5.0, 2.6 Hz, 2H), 1.63 – 1.45 (m, 5H), 1.35 – 1.19 (m, 13H), 1.13 (s, 3H), 1.02 (s, 9H), 0.97 (s, 3H), 0.90 (dt, *J* = 21.0, 8.0 Hz, 23H), 0.80 (d, *J* = 6.3 Hz, 3H), 0.59 – 0.54 (m, 6H), 0.53 – 0.47 (m, 6H).

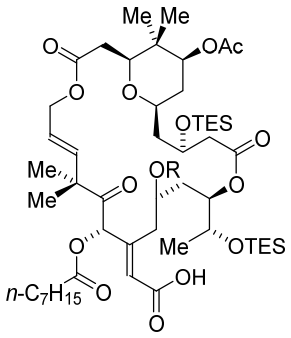
^{13}C NMR (151 MHz, $CDCl_3$) δ 204.9, 172.2, 171.8, 170.6, 170.6, 167.5, 138.1, 136.1, 136.0, 133.5, 133.2, 129.9, 127.6, 127.6, 125.0, 80.7, 73.8, 73.3, 72.1, 68.2, 67.8, 64.9, 63.7, 60.5, 49.9, 43.8, 43.4, 37.2, 35.1, 34.3, 33.5, 31.6, 29.7, 29.0, 28.9, 27.1, 25.0, 24.5, 23.8, 22.7, 22.6, 22.3, 21.1, 19.3, 18.1, 14.04, 13.7, 6.9, 4.9, 4.8.

FTIR (neat) υ 2954, 2929, 2875, 2852, 1739, 1695, 1647, 1458, 1377, 1240, 1166, 1105, 1084, 1007, 740, 703 cm^{-1}.

HRMS (ESI) Calcd. for $C_{68}H_{108}O_{15}Si_3Na$ $[M+Na]^+$: 1271.6894, Found: 1271.6882.

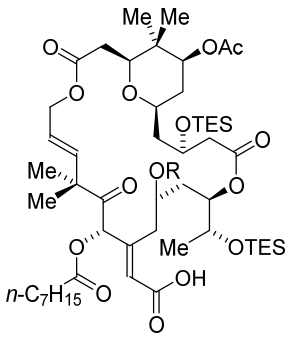
$[\alpha]^{32.5}_D$: +2.67° (c = 0.5, $CHCl_3$).

TLC (SiO_2): R_f = 0.60 (30% ethyl acetate in hexanes).


Me Me
OAc
O
O
O
OTES
O
Me
Me
O
OR
O
Me
OTES
O
OH
n-C7H15
O
O

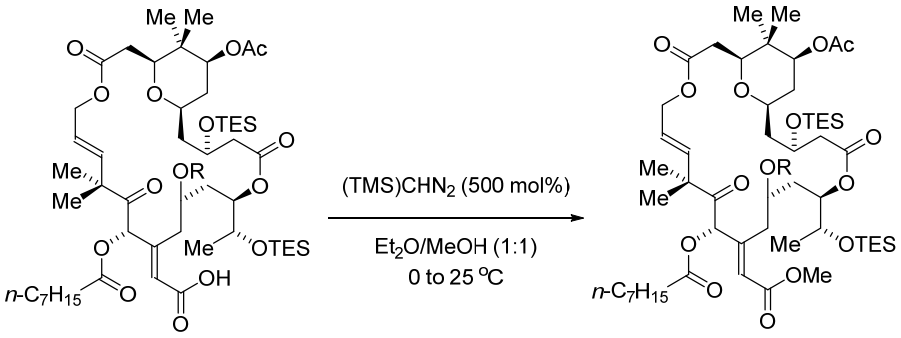
13, R = TBDPS

8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Me Me
OAc
O
O
O
OTES
O
Me
OR
Me
O
O
Me
OTES
O
OH
n-C7H15
O
O

13, R = TBDPS

230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



13, R = TBDPS

13a, R = TBDPS
85% yield

Carboxylic acid **13** (27.2 mg, 0.022 mmol, 100 mol%) was dissolved in 5.6 mL of 1:1 MeOH/Et_2O, put under argon, and cooled to 0 °C. TMS-diazomethane (54 µL of a 2M solution in hexanes, 0.11 mmol, 500 mol%) was added dropwise over 2 minutes. The reaction was stirred for 5 minutes at 0 °C then at room temperature for an additional 1.5 hours. The reaction was diluted with pH 7 buffer and poured into brine. The layers were separated and the aqueous was extracted three times with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified via flash column chromatography (5 → 10% EtOAc/Hexanes) to yield ester **13a** (23.5 mg, 85%) as a thin film.

Characterization data for ester **13a**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.59 (m, 4H), 7.36 (dtd, *J* = 14.5, 8.3, 7.6, 6.4 Hz, 6H), 5.93 (s, 1H), 5.80 – 5.72 (m, 2H), 5.65 (dt, *J* = 16.2, 6.1 Hz, 1H), 4.81 (dt, *J* = 8.3, 4.3 Hz, 1H), 4.69 (dd, *J* = 11.6, 4.7 Hz, 1H), 4.63 (dd, *J* = 13.1, 6.0 Hz, 1H), 4.55 (dd, *J* = 13.1, 5.6 Hz, 1H), 4.32 – 4.13 (m, 2H), 3.83 – 3.74 (m, 1H), 3.61 (s, 3H), 3.60 – 3.57 (m, 1H), 3.52 (t, *J* = 10.4 Hz, 1H), 3.27 (dd, *J* = 13.2, 5.1 Hz, 1H), 2.56 – 2.31 (m, 5H), 2.21 (dt, *J* = 15.7, 7.7 Hz, 1H), 2.17 – 2.05 (m, 1H), 2.07 (s, 3H) 2.01 (dt, *J* = 14.3, 5.1 Hz, 1H), 1.81 – 1.70 (m, 1H), 1.70 – 1.60 (m, 1H), 1.57 – 1.41 (m, 8H), 1.38 (s, 3H), 1.25 (s, 12H), 1.11 (s, 3H), 0.99 (s, 9H), 0.95 – 0.84 (m, 20H), 0.80 (d, *J* = 6.3 Hz, 3H), 0.61 – 0.48 (m, 12H).

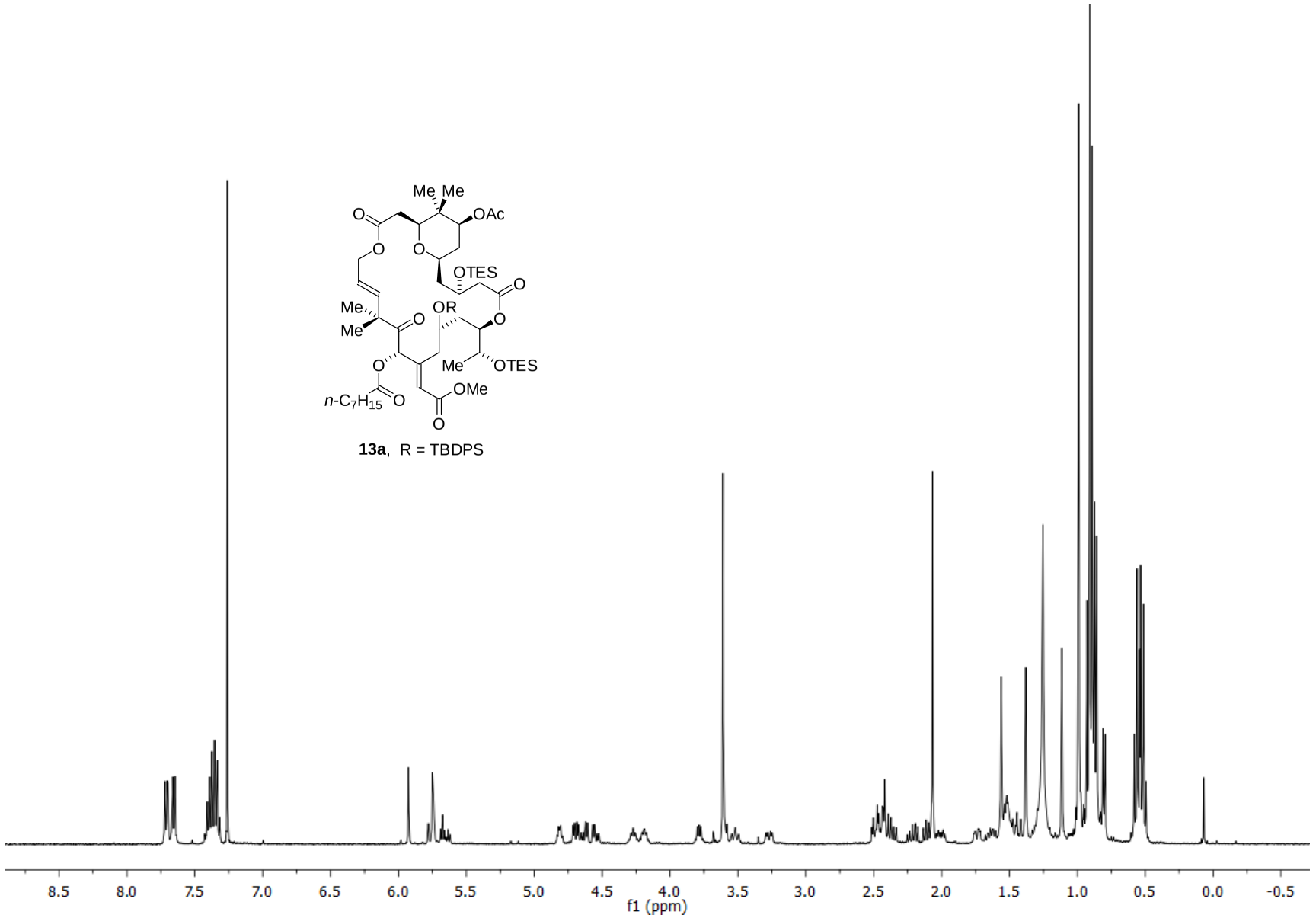
^{13}C NMR (151 MHz, $CDCl_3$) δ 206.7, 172.2, 171.7, 170.9, 170.6, 165.4, 150.5, 137.9, 136.2, 135.9, 134.1, 134.0, 129.6, 129.5, 127.5, 127.4, 124.9, 121.2, 80.5, 77.2, 76.3, 73.8, 73.1, 71.7, 67.8, 67.7, 65.2, 51.1, 50.0, 44.3, 43.5, 37.9, 37.2, 37.1, 35.3, 34.3, 33.5, 31.6, 29.7, 29.0, 28.9, 27.1, 24.8, 24.5, 24.4, 22.6, 22.4, 21.1, 19.3, 17.7, 14.1, 13.7, 6.9, 6.8, 5.0, 4.8.

FTIR (neat) υ 2954, 2932, 2875, 2857, 1731, 1461, 1377, 1241, 1166, 1105, 1085, 1009, 739, 703 cm^{-1}.

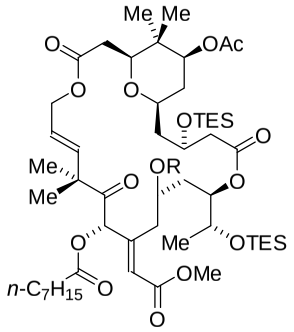
HRMS (ESI) Calcd. for $C_{69}H_{110}O_{15}Si_3Na$ $[M+Na]^+$: 1285.7050, Found: 1285.7064.

$[\alpha]^{32.3}_D$: +42.11° (c = 0.15, $CHCl_3$).

TLC (SiO_2): R_f = 0.54 (20% ethyl acetate in hexanes).

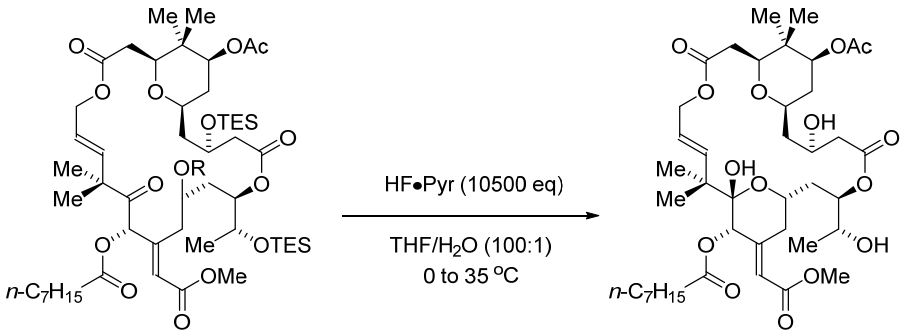

Me Me
OAc
O
O
O
OTES
O
Me
Me
OR
O
O
O
Me
OTES
n-C7H15
O
OMe
O
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

13a, R = TBDPS


Me Me
OAc
O
O
O
OTES
O
Me
Me
OR
O
O
Me
OTES
O
OMe
n-C7H15
O
O

13a, R = TBDPS

240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



13a, R = TBDPS

WN-1
70% yield

A polyethylene vial was charged with the protected bryolog **13a** (22 mg, 0.0174 mmol, 100 mol%). The compound was dissolved in THF (17.4 mL) and water (176 µL). The reaction was put under argon and cooled to 0 °C. To this solution was added HF·pyridine (4.5 mL of a commercial 70% HF solution, 182 mmol, 10,500 equiv.) over 50 minutes. After the addition was complete, the solution was stirred for an additional 1 hour at this temperature. The cooling bath was removed and the reaction was stirred at room temperature for 24 hours. The reaction was then warmed to 35 °C for an additional 54 hours. The reaction was then cooled to 0 °C and diluted with pH 7 buffer. The reaction was quenched at this temperature with saturated $NaHCO_{3(aq)}$. The layers were separated and the aqueous phase was extracted three times with ethyl acetate. The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified via flash column chromatography (30 → 50% EtOAc/Hexanes). The fractions containing **WN-1** were concentrated and further purified via reverse phase HPLC with a Phenomenex Gemini C18 (10 µm particle size, 300Å pore size), 21.2 mm diameter column using 85% MeCN/H_2O containing 0.1% TFA as the eluent (flow rate of 8 mL/min) to yield **WN-1** (retention time 14 min) as a white solid (9.5 mg, 70%).

Characterization data for bryolog **WN-1**

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.04 (dd, *J* = 15.8, 1.4 Hz, 1H), 5.98 (d, *J* = 2.0 Hz, 1H), 5.50 (ddd, *J* = 15.8, 10.5, 3.2 Hz, 1H), 5.31 (ddd, *J* = 12.2, 5.0, 2.8 Hz, 1H), 5.13 (s, 1H), 4.82 (dd, *J* = 11.7, 10.6 Hz, 1H), 4.66 – 4.60 (m, 2H), 4.53 (ddd, *J* = 11.7, 3.1, 1.7 Hz, 1H), 4.28 (td, *J* = 10.9, 5.5 Hz, 1H), 4.02 – 3.92 (m, 1H), 3.86 – 3.70 (m, 4H), 3.68 (s, 3H), 3.59 (t, *J* = 6.7 Hz, 1H), 2.49 – 2.37 (m, 4H), 2.31 (td, *J* = 7.7, 5.6 Hz, 2H), 2.12 – 1.97 (m, 5H), 1.87 – 1.74 (m, 2H), 1.71 (d, *J* = 7.2 Hz, 1H), 1.61 (q, *J* = 7.3 Hz, 1H), 1.53 – 1.42 (m, 1H), 1.33 – 1.24 (m, 11H), 1.23 (d, *J* = 6.5 Hz, 3H), 1.17 (s, 3H), 1.04 (s, 3H), 0.90 (s, 3H), 0.89 – 0.85 (m, 6H).

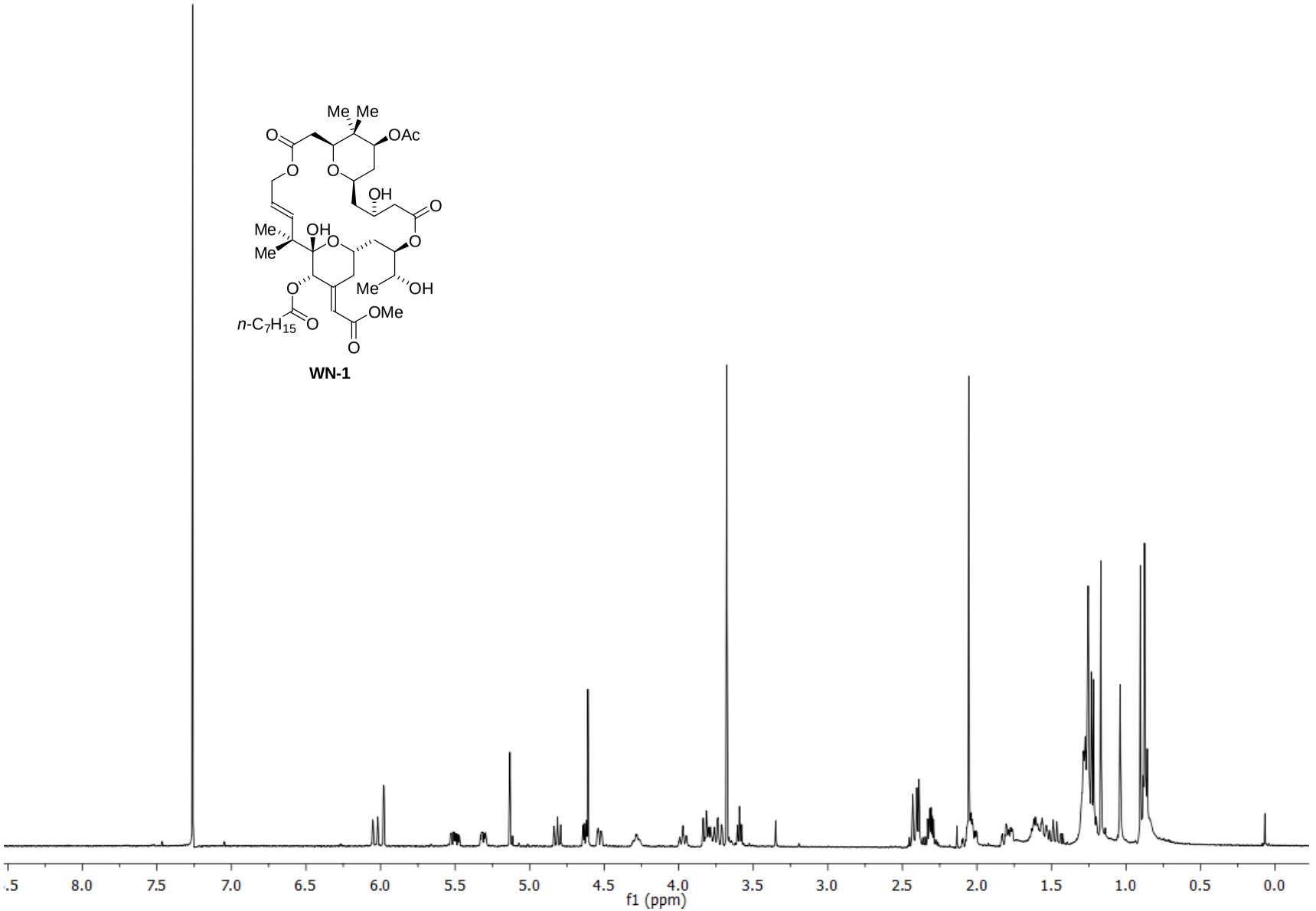
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 171.9, 171.3, 170.5, 166.9, 151.5, 142.9, 122.1, 119.9, 98.9, 82.2, 76.0, 74.0, 73.6, 73.1, 70.1, 69.0, 67.2, 64.9, 51.1, 45.1, 42.3, 39.6, 37.4, 36.0, 35.5, 34.6, 33.8, 31.6, 29.7, 29.0, 28.9, 24.7, 24.3, 22.6, 22.3, 21.0, 20.0, 19.4, 14.0, 13.5.

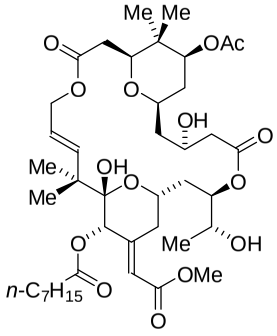
FTIR (neat) υ 3497, 2953, 2855, 1734, 1668, 1459, 1364, 1240, 1154, 1076 cm^{-1}.

HRMS (ESI) Calcd. for $C_{41}H_{64}O_{15}Na$ $[M+Na]^+$: 819.4143, Found: 819.4122.

$[\alpha]^{32.7}_D$: +13.33° (c = 0.2, $CHCl_3$).

TLC (SiO_2): R_f = 0.24 (60% ethyl acetate in hexanes).

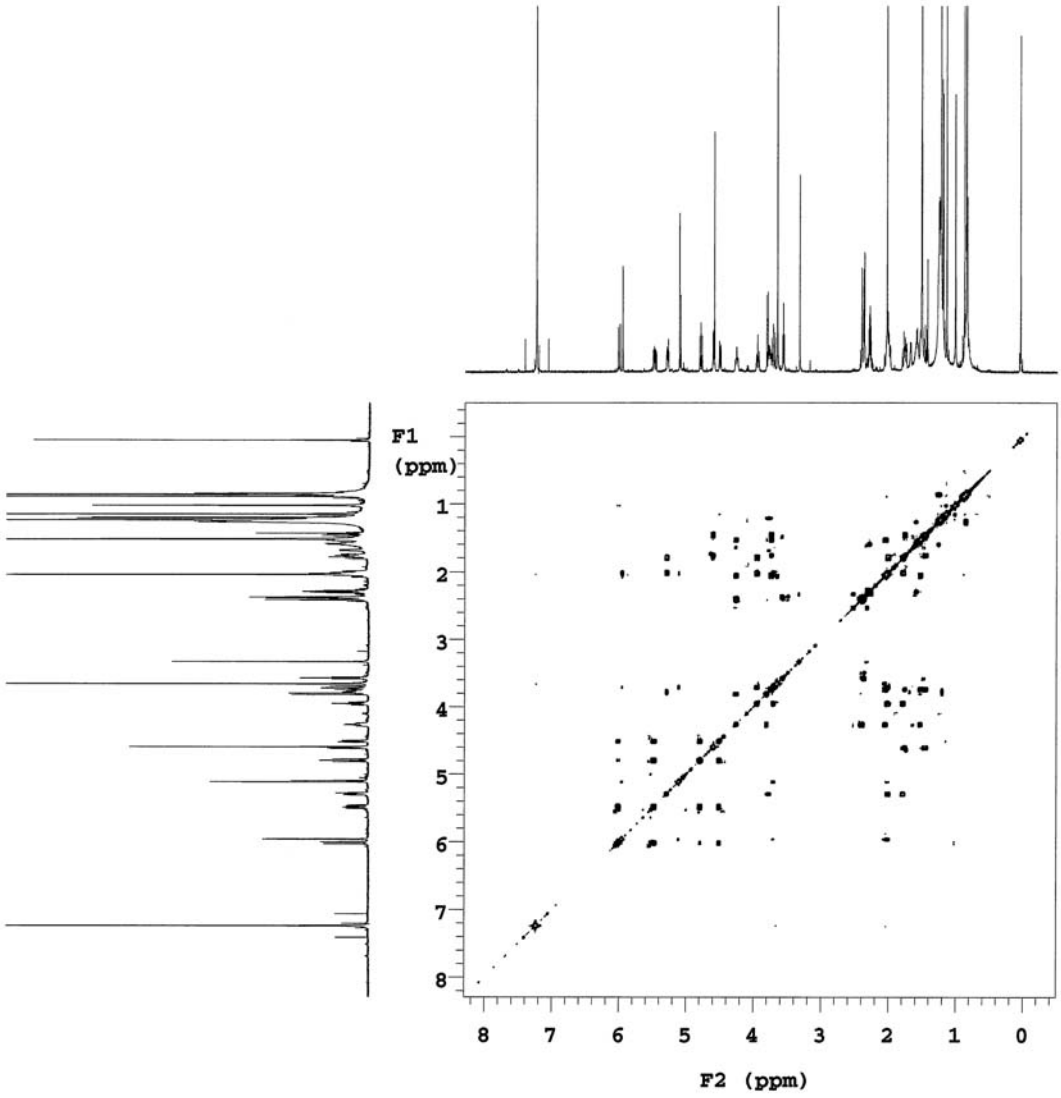

Me Me
OAc
O
O
O
OH
O
Me
OH
O
O
Me
Me
OH
O
n-C7H15
O
OMe
O
WN-1
.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

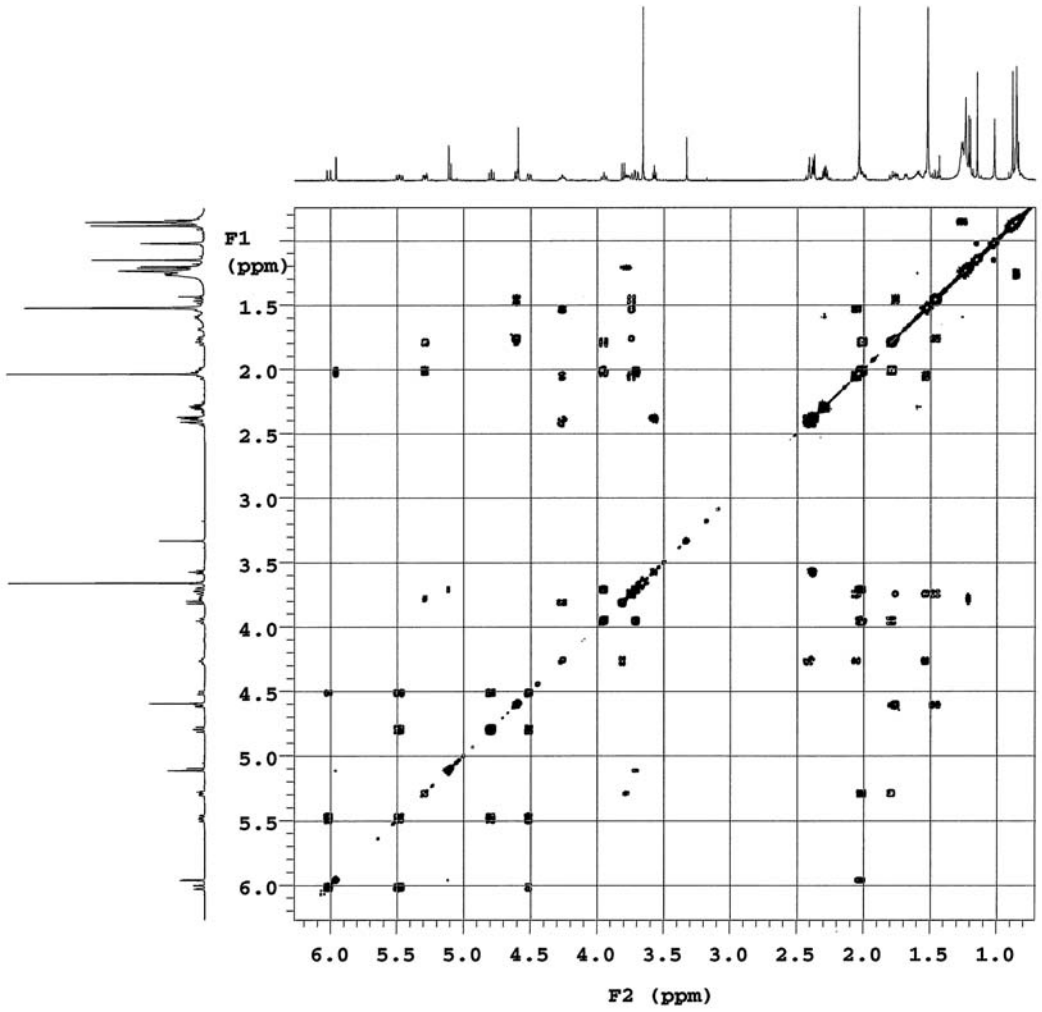

Me Me
OAc
O
O
O
OH
O
Me
OH
O
O
Me
Me
OH
O
OMe
n-C7H15
O
O

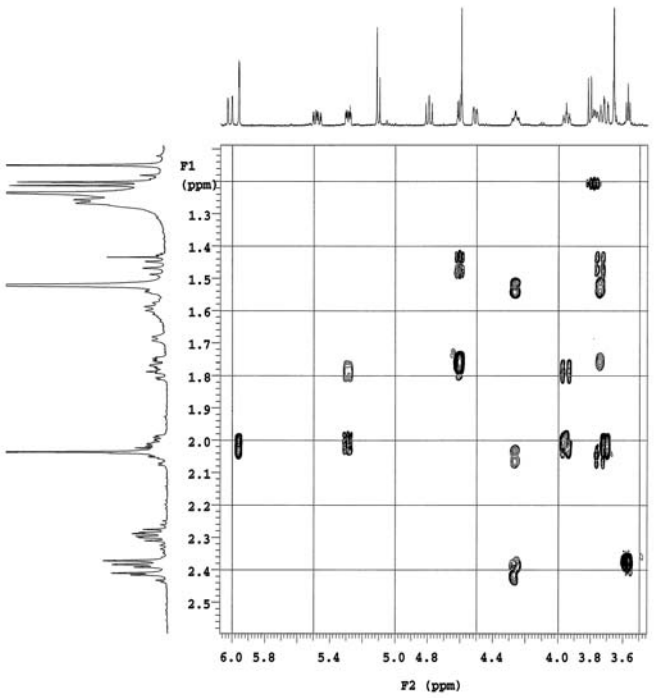
WN-1

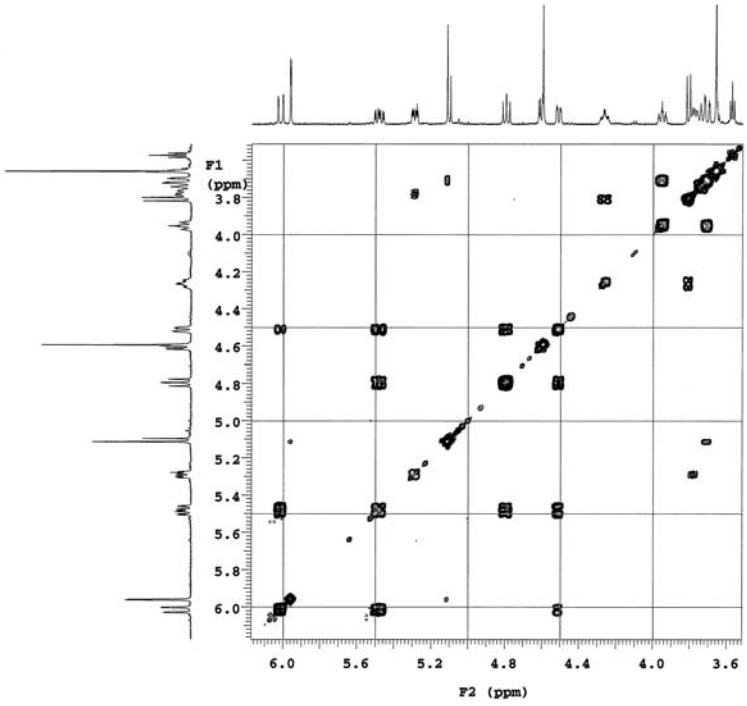
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

gCOSY Spectrum of WN-1 with Expansions



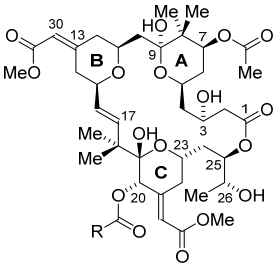

F1
(ppm)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
F2 (ppm)


F1
(ppm)
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
2.1
2.2
2.3
2.4
2.5
6.0 5.8
5.4
5.0 4.8
4.4
4.0 3.8 3.6
F2 (ppm)


F1
(ppm)
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8
6.0
6.0
5.6
5.2
4.8
4.4
4.0
3.6
F2 (ppm)

A. Comparison of the Spectral Data for Bryostatin 1, WN-1, Merle 42 and Merle 43

(chemical shifts for the proton geminal to the acylated C25 or C26 oxygen are highlighted in blue)



Bryostatin 1, R = $(CH)_4(CH_2)_2Me$

K_i = 0.48 nM[a]

Correlations from: Keck, G. E.; Poudel, Y. B.; Cummins, T. J.; Rudra, A.; Covel, J. A. *J. Am. Chem. Soc.* **2011**, *133*, 744.

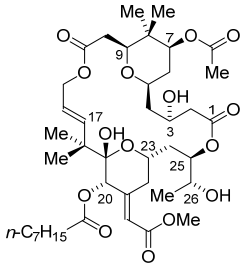
H27 1.24 ppm (d, *J* = 6.4 Hz, 3H)

H26 3.78 ppm (m, 1H)

H25 5.17 ppm (m, 1H)

H24a 1.83 ppm (ddd, *J* = 13.8, 11.6, 2.9 Hz, 1H)

H24b 1.99 ppm (m, 1H)



WN-1

K_i = 16.6 nM[a]

PMA-like behavior observed.

Correlations from 1H NMR and gCOSY of **WN-1**

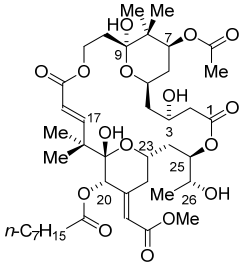
H27 1.23 ppm (d, *J* = 6.5 Hz, 3H) couples to H26

H26 3.78 ppm (m, 1H) couples to H27, H25

H25 5.31 ppm (ddd, *J* = 12.2, 5.0, 2.8, 1H) couples to H26, H24a, H24b

H24a within 1.87-1.74 ppm (m, 2H) couples to H24b, H25

H24b within 2.12-1.97 ppm (m, 5H) couples to H24a, H25



Merle 42

K_i = 0.75 nM

PMA-like behavior observed.

Best estimates from 1H NMR and gCOSY from: Kraft, M. B. Ph.D. Dissertation, The University of Utah, 2011.

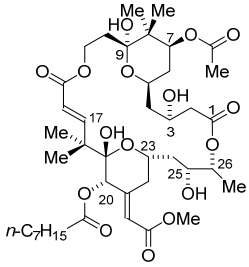
H27 1.22 ppm (d, *J* = 6.4 Hz, 3H) couples to H26

H26 3.86-3.80 ppm (m, 1H) couples to H27

H25 5.14 ppm (ddd, *J* = 11.5, 5.5, 3.0 Hz, 1H) couples H24a

H24a within 1.87-1.78 ppm (m, 2H) couples to H24a, H25

H24b within 1.99-1.92 ppm (m, 3H) couples to H24a



Merle 43

K_i = 13.8 nM

PMA-like behavior observed.

Best estimates from 1H NMR and gCOSY from: Kraft, M. B. Ph.D. Dissertation, The University of Utah, 2011.

H27 1.24 ppm (d, *J* = 6.4 Hz, 3H) couples to H26

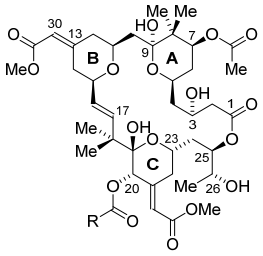
H26 5.04 ppm (dq, *J* = 6.6, 6.6 Hz, 1H); couples to H27

H25 3.87 ppm (dd, *J* = 7.0, 7.0 Hz, 1H) couples to H24a

H24a within 1.73-1.47 ppm (m, 7H) couples to H25, H24b

H24b within 1.96-1.90 ppm (m, 3H) couples to H24a

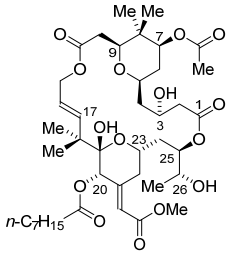
B. Comparison of the Spectral Data for Bryostatin 1 and WN-1



Bryostatin 1, R = $(CH)_4(CH_2)_2Me$

K_i = 0.48 nM[a]

Correlations from: Keck, G. E.; Poudel, Y. B.; Cummins, T. J.; Rudra, A.; Covel, J. A. *J. Am. Chem. Soc.* **2011**, *133*, 744.



WN-1

K_i = 16.6 nM[a]

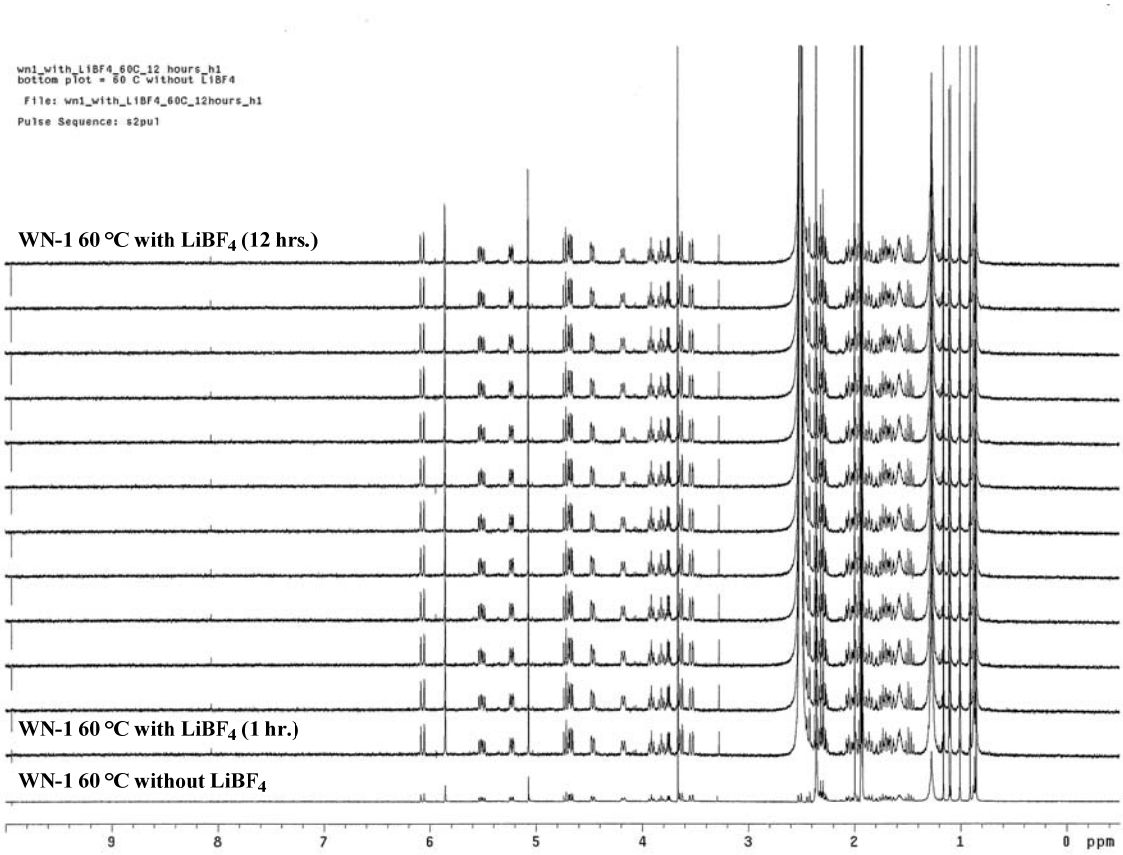
PMA-like behavior observed.

Correlations from 1H NMR and gCOSY of **WN-1**

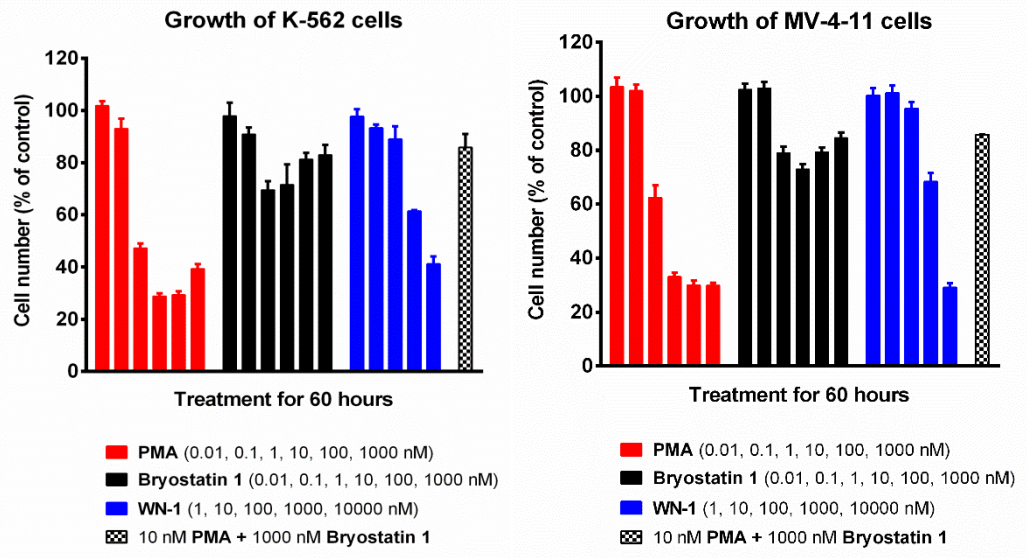
H27 1.24 ppm (d, *J* = 6.4 Hz, 3H)	H27 1.23 ppm (d, *J* = 6.5 Hz, 3H) couples to H26
H26 3.78 ppm (m, 1H)	H26 3.78 ppm (m, 1H) couples to H27, H25
H25 5.17 ppm (m, 1H)	H25 5.31 ppm (ddd, *J* = 12.2, 5.0, 2.8 Hz, 1H) couples to H26, H24a, H24b
H24a 1.83 ppm (ddd, *J* = 13.8, 11.6, 2.9 Hz, 1H)	H24a within 1.87-1.74 ppm (m, 2H) couples to H24b, H25
H24b 1.99 ppm (m, 1H)	H24b within 2.12-1.97 ppm (m, 5H) couples to H24a, H25
H23 4.02 ppm (tt, *J* = 11.3, 2.4 Hz, 1H)	H23 4.02 - 3.92 ppm (m, 1H)
$H22_{eq}$ 3.69 ppm (m, 1H)	$H22_{eq}$ within 3.86 - 3.70 ppm (m, 4H)
$H22_{ax}$ 2.06 ppm (m, 1H)	$H22_{ax}$ within 2.12 - 1.97 ppm (m, 5H)
H20 5.18 ppm (s, 1H)	H20 5.13 ppm (s, 1H)
H17 5.78 ppm (d, *J* = 15.6 Hz, 1H)	H17 6.04 (dd, *J* = 15.8, 1.4 Hz, 1H)
H16 5.32 ppm (dd, *J* = 15.7, 8.5 Hz, 1H)	H16 5.50 (ddd, *J* = 15.8, 10.5, 3.2 Hz, 1H)
H15 4.09 ppm(ddd, *J* = 11.0, 8.5, 2.5 Hz, 1H)	H15 4.82 (dd, *J* = 11.7, 10.6 Hz, 1H) and 4.53 (ddd, *J* = 11.7, 3.1, 1.7 Hz, 1H)
H10a 1.66 ppm (d, *J* = 14.9 Hz, 1H)	H10a within 2.49 - 2.37 ppm (m, 4H)
H10b 2.06 ppm (m, 1H)	H10b within 2.49 - 2.37 ppm (m, 4H)
H7 5.19 ppm (m, 1H)	H7 within 4.66 - 4.60 ppm (m, 2H)
$H6_{eq}$ 1.72 ppm (ddd, *J* = 12.3, 4.6, 2.6 Hz, 1H)	$H6_{eq}$ within 1.87 - 1.74 ppm (m, 2H)
$H6_{ax}$ 1.48 ppm (q, *J* = 11.7 Hz, 1H)	$H6_{ax}$ 1.53 - 1.42 ppm (m, 1H)
H5 4.21 ppm (tt, *J* = 11.6, 2.3 Hz, 1H)	H5 within 3.86 - 3.70 ppm (m, 4H)
H4a 1.55 ppm (ddd, *J* = 14.8, 3.6, 3.1 Hz, 1H)	H4a 1.61 ppm (q, *J* = 7.3 Hz, 1H)
H4b 2.02 ppm (m, 1H)	H4b within 2.12 - 1.97 ppm (m, 5H)
H3 4.12 ppm (m, 1H)	H3 4.28 ppm (td, *J* = 10.9, 5.5 Hz, 1H)
H2b 2.53 ppm (dd, *J* = 12.2, 12.0 Hz, 1H)	H2b within 2.49 - 2.37 ppm (m, 4H)
H2a 2.42 ppm (dd, *J* = 12.2, 2.1 Hz, 1H)	H2a within 2.49 - 2.37 ppm (m, 4H)

C. Treatment of WN-1 with $LiBF_4$

To an NMR tube containing **WN-1** (1.5 mg, 0.0019 mmol, 100 mol%) in a solution of CD_3CN:D_2O (672 µL:28 µL) was added $LiBF_4$ (4.4 mg, 0.047 mmol, 2500 mol%). The tube was sealed, placed in a 500 mHz NMR instrument and heated to 60 °C for 12 hours. A ^1H NMR of the sample was collected every hour over a 12 hour period, during which time no detectable change of **WN-1** was observed. The results of this experiment are shown below.



III. Supplemental Figure 1



Supplemental Figure 1. Inhibition of growth of K562 and MV-4-11 cells by PMA, bryostatin 1, or **WN-1**.

IV. Biological Methods

A. Measurement of Binding Affinity of WN-1 to Human PKCα

Protein kinase C alpha (hPKCα), human, was purchased from Invitrogen Life Technologies, (Madison, WI). [20-^{3}H]Phorbol 12, 13-dibutyrate (13.5 Ci/mmole) was obtained from Perkin-Elmer Life Sciences, Inc. (Boston, MA) as a custom synthesis. Non-radioactive phorbol 12, 13-dibutyrate was from LC Laboratories (Woburn, MA). L-alpha-phosphatidylserine (PS) was from Avanti Polar Lipids, Inc. (Alabaster, Al). γ-Globulins (G5009) were from Sigma-Aldrich, Inc. (St. Louis MO). Polyethylene glycol 6000 was purchased from EMD Chemicals, Inc. (Gibbstown, NJ). Sarstedt 1.5 ml (72.692) microfuge tubes were from VWR International LLC, (Radnor, PA). TX-100 was from RPI Corp. (Mount Prospect, IL).

The binding affinity of **WN-1** to hPKCα was determined by its competition of [20-^{3}H]PDBu binding using the poly(ethylene) glycol precipitation assay[3] with minor modifications. Tubes contained 250 µl of 50 mM Tris-HCl (pH 7.4), 0.1 mM $CaCl_2$, 100 µg/ml phosphatidylserine, 4 mg/ml bovine IgG, 2.5 nM [^{3}H]PDBu, 0.003% TX-100 and a series of increasing concentrations of **WN-1**. In each assay, binding at each **WN-1** concentration was determined in triplicate and a fourth tube included 20 µM non-radioactive PDBu for measuring non-specific binding. Concentrations of **WN-1** represented a series of half-log steps from 0.3 nM to 3000 nM. Control binding was determined in the absence of **WN-1**. The tubes were incubated for 5 minutes at 37°C. The samples were then chilled on ice for 10 minutes, and 200 µl of 35% poly(ethylene) glycol in 50 mM Tris-HCl (pH 7.4) was added and the tubes were vigorously vortexed. The tubes were incubated on ice for an additional 10 minutes and then centrifuged at 4°C. A 100 µL aliquot of the supernatant was removed for the determination of the free [^{3}H]PDBu concentration, and the pellet was carefully dried. The tip of the tube was cut off, and the pellet was counted in a scintillation counter to determine the total bound [^{3}H]PDBu. Specific binding was calculated as the difference between the total and the nonspecific binding. The K_i was calculated from the inhibitory binding curve as described by Cheng and Prusoff.[4] The experiment was performed in triplicate to yield a mean ± SE for the K_i. The analysis was subsequently repeated on a separate batch of **WN-1**, with triplicate determinations yielding a similar value.

B. Stability of WN-1 Under Simulated Conditions for *K*$_i$ Assay

A microfuge tube containing 1.5 mg of **WN-1** in a 1.0 mL solution of 50 mM Tris-HCl (pH 7.4), 0.1 mM $CaCl_2$, 100 µg/ml phosphatidylserine, 4 mg/ml bovine IgG, and 0.003% TX-100

[3] Lewin, N.E.; Blumberg, P. M. *Methods Mol. Biol*. **2003**, *233*, 129.
[4] Cheng Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

was vigorously vortexed followed by incubating for 5 minutes at 37°C. The sample was then chilled on ice for 10 minutes, then vigorously vortexed, placed on ice for an additional 10 minutes and centrifuged at 4°C for 15 minutes. The solution was then extracted three times with ethyl acetate and the combined organic layers were washed with brine (6 times), dried over Na_2SO_4, filtered, and concentrated to give a pure sample of **WN-1** without further purification. The purity of the sample was confirmed by ^1H NMR on 500 MHz.

C. Growth Inhibition and Attachment:

U937, K562, MV-4-11 and Toledo cells (from ATCC, Manassas, VA) were cultured in RPMI-1640 medium supplemented with 10 % FBS (ATCC, Manassas, VA) and 2 mM glutamine (Life Technologies, Carlsbad, CA). Cells were plated in 35 mm dishes (BD Biosciences, Bedford, MA) at a density of 1 x 10^5cells/ml (2 x 10^5 cells/ml for Toledo cells) and 24 hours later treated with different concentrations of the compounds or DMSO (final concentration 0.1 % for all treatments). After 60 hours, the number of cells (size range 7-24 μm) was counted using a Beckman particle counter (Beckman Coulter Inc., Fullerton, CA). For U937 cells the number of unattached cells present in the supernatant and the number of attached cells (determined after trypsinization) were counted separately; the number of attached cells was expressed as percent of total cells. Values presented are the mean ± SE of 3 independent experiments.

D. Real Time qPCR Analysis:

U937 cells (4 ml of 150,000 /ml cells cultured in 60 mm dishes) were treated 24 hours after plating with the indicated compounds or DMSO (final DMSO concentration was 0.1 % for all treatments). Cells were collected by centrifugation (1500 x g for 5 min) and RNA was isolated from cell pellets with TRIzol reagent following the manufacturer's protocol (Invitrogen, Carlsbad, CA). For cDNA synthesis, 1.5 μg of total RNA was reverse transcribed using iScript

Advanced cDNA Synthesis Kit (BIO-RAD, Hercules CA). Real time PCR was performed on a MyiQ instrument (BIO-RAD, Hercules, CA) in a volume of 20 µl using iQ SYBR Green Supermix from BIO-RAD (Hercules, CA) with 150 times diluted cDNA. The primers used were predesigned Quantitect primers from Qiagen (Valencia, CA). Relative gene expression levels were calculated using the $2^{-(\Delta Ct)}$ formula, where ΔCt represents the cycle difference corrected for GAPDH, used as internal control. The data are presented as fold change in gene expression normalized to GAPDH and relative to the DMSO treated control. The efficiency of the qPCR reaction for GAPDH and TNF alpha was between 108.8 and 104.8 %, respectively, when tested on serially diluted (1:5) "universal" RNA samples prepared to contain all transcripts of interest. Values represent the mean ± SE of triplicate independent experiments.

V. Modeling Results and Methods

A. Discussion of Results

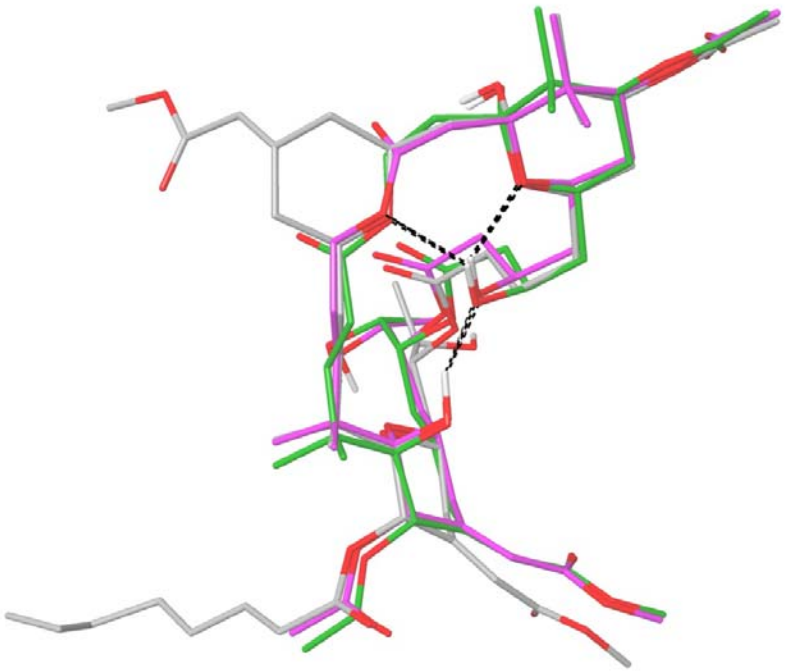
*Molecular modeling of **WN-1** and **Merle 42** conformation and PKC C1 domain binding.*

To analyze the effect of replacing the B-ring with an ester linkage on the overall conformation of the macrolide ring, we performed a thorough conformational search of **WN-1** and **Merle 42** in octanol solvent. The lowest-energy conformation found in both cases retained a strong similarity to the crystal conformation of bryostatin 1[5] (Supplemental Figure 2). The A- and C-rings can be overlaid nearly exactly and the ether oxygen in the ester linkage aligns with the pyran oxygen in the bryostatin B-ring. This allows the internal hydrogen bonding structure of bryostatin to be preserved in both of these *seco*-B-ring analogues.

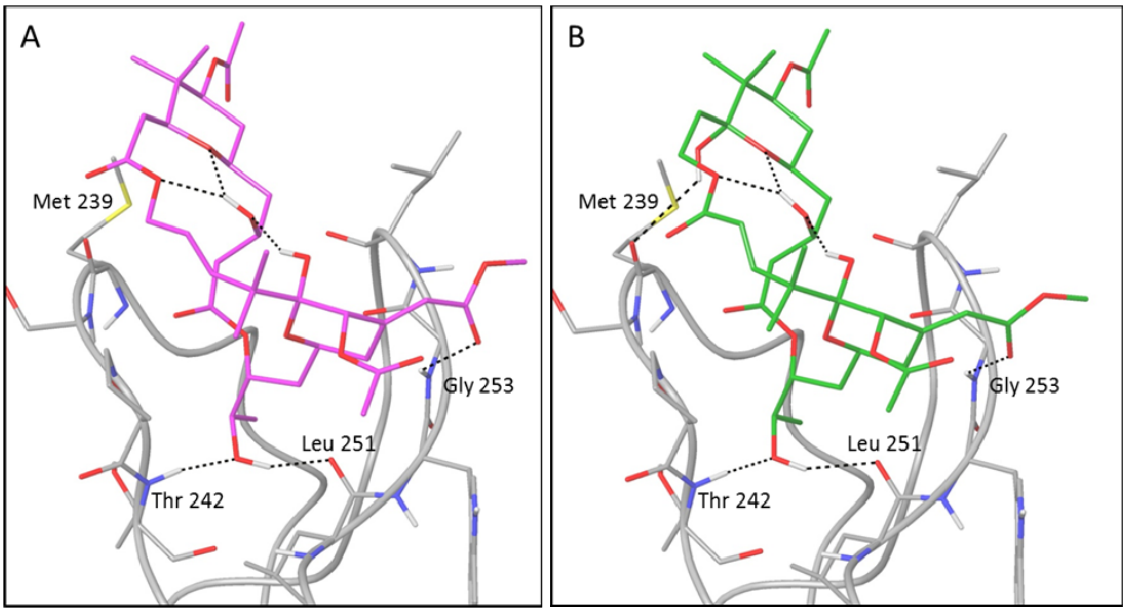
We then docked **WN-1** and **Merle 42** into the crystal structure of the C1b domain of PKCδ[6] and found, as expected based on the conformational analysis, that both analogues reproduce the binding mode of bryostatin,[7] with the C26 hydroxyl hydrogen bonding to the backbone at Thr 242 and Leu 251, and the C-ring methoxycarbonyl group hydrogen bonding to Gly 253. The C9 hydroxyl in **Merle 42** forms an additional hydrogen bond to the backbone carbonyl of Met 239.

[5] Pettit, G.; Herald, C.; Doubek, D.; Herald, D.; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6846.
[6] Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917.
[7] Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Peach, M. L.; Blumberg, P. M. *Angew. Chem. Int. Ed Engl.* **2010**, *49*, 4580.

Although the position of the carbonyl oxygen in the ester linkage varies between **WN-1** and **Merle 42**, in both docked structures it remains solvent exposed and does not form any interactions with the C1 domain (Supplemental Figure 3).



Supplemental Figure 2. An overlay of the crystal structure of bryostatin 1 (grey) with low-energy conformers of **WN-1** (magenta), and **Merle 42** (green). Intramolecular hydrogen bonds are shown as black dashed lines.

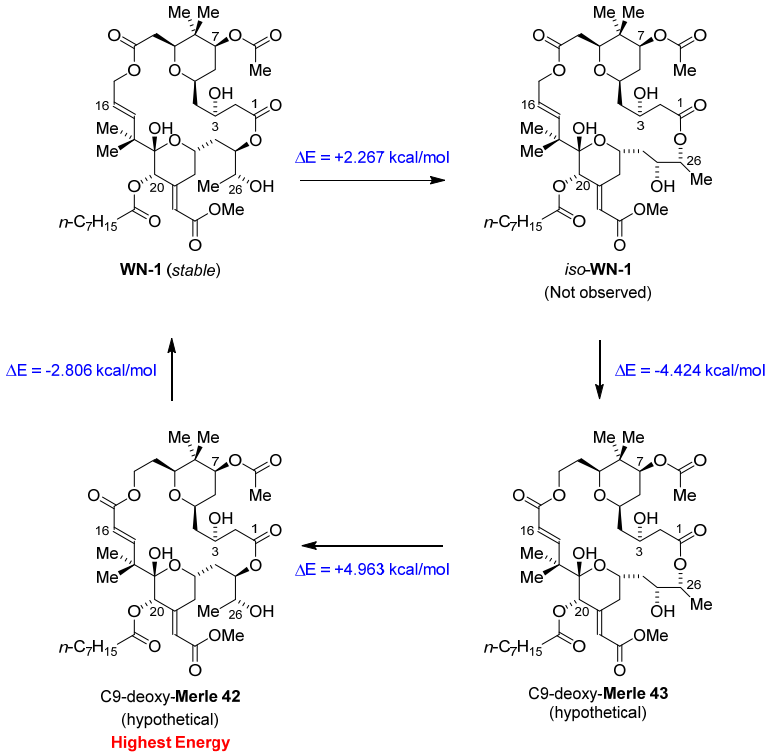


Supplemental Figure 3. Binding mode of **WN-1** (A) and **Merle 42** (B) in the PKCδ C1b domain. Hydrogen bonds are indicated by dashed black lines.

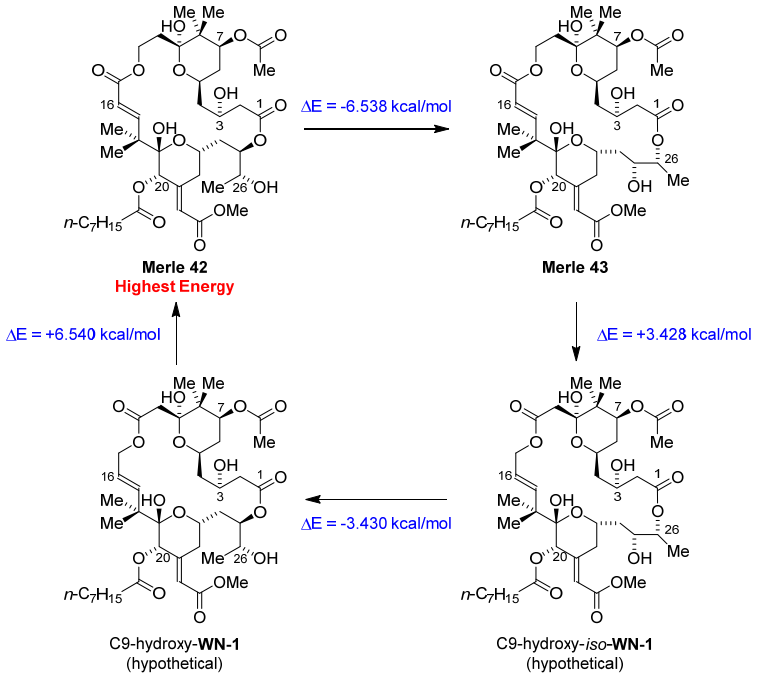
The conformational analysis and docking results suggest that the ~20-fold difference in binding affinity between **WN-1** and **Merle 42** is not due to any significant change in conformation or loss of favorable interactions with the PKC C1 domain, although it is possible that in the absence of the B-ring the C9 hydroxyl has a much stronger effect on binding than it does in the context of the full A+B ring structure.[7]

Energy of ring isomerization reaction.

We calculated the energies of the two ring expansion reactions, i.e. the observed conversion of **Merle 42** into **Merle 43** and the theoretically equivalent conversion of **WN-1** into *iso*-**WN-1**, using the lowest-energy conformer for each compound. Geometry optimizations for each structure were run at the B97-D3/6-31G(d) level and subsequent single-point energies were calculated at the ωB97X-D/6-311G(2d,2p) level. The reaction energy for **Merle 42** → **Merle 43** was -6.54 kcal/mol, whereas the energy for **WN-1** → *iso*-**WN-1** was 2.27 kcal/mol, confirming that the rearrangement of **Merle 42** into **Merle 43** is energetically favorable while the equivalent rearrangement of **WN-1** into *iso*-**WN-1** is not. For further comparison, the energies for various C9-deoxy and C9-hydroxy *seco*-B-ring analogues were calculated. The results from these studies are shown in Supplemental Figures 4 and 5.



Supplemental Figure 4. Relative Energies for C9-deoxy *seco*-B-ring Analogues



Supplemental Figure 5. Relative Energies for C9-hydroxy *seco*-B-ring Analogues

It is interesting to note that in both cases (Supplemental Figures 4 and 5) **Merle 42**/C9-deoxy-**Merle 42** is the highest in energy of the four isomers, and the presence of the C9 hydroxyl seems to worsen the ring strain energy. This may be because in **Merle 43** and the C9-hydroxy-**WN-1s** the C9-hydroxyl can form an intramolecular hydrogen bond, whereas in **Merle 42** it does not.

B. Modeling Methods

Conformational Searching: The initial structures for **WN-1**, **Merle 42**, *iso*-**WN-1**, and **Merle 43** were built based on the crystal structure of bryostatin from the Cambridge Structural Database (reference code BOKKIV).[5] The acyl tail in each structure was truncated to a methyl group to reduce the size of the conformational space to be searched. All searches were performed using mixed torsional/large-scale low-mode sampling in MacroModel[8,9,10] with the OPLS 2005 forcefield[11] in octanol implicit solvent. During the searches torsions were varied for 10,000 steps with enhanced sampling, but the chiral centers and double bonds were restricted to their crystal

[8] *MacroModel version 10.1*; Schrödinger, LLC: New York, NY, 2013.
[9] Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.
[10] Kolossváry, I.; Keserü, G. M. *J. Comput. Chem.* **2001**, *22*, 21.
[11] Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.

conformations. Low mode displacements were between 3 and 18 Å. After each step the resulting structure was energy minimized to a gradient convergence of 0.05. If the minimized structure was within an energy cutoff of 10 kcal/mol of the global minimum, it was then compared to previously stored structures and either kept as a unique conformer or rejected as a duplicate, using a 0.75 Å RMSD cutoff to the heavy atoms in the central macrolide ring structure. A set of 20 low-energy conformers for each structure was passed on to the docking program, and a smaller set of two or three conformers was passed on for quantum mechanical calculations.

Docking: The crystal structure of the C1b domain of PKC-δ was prepared for docking by adding hydrogen atoms and deleting the phorbol-13-acetate ligand. This was saved to a separate file to be used as a template for the similarity constraint (see below). Docking was done using the program GOLD, version 5.2.2,[12] which uses a genetic algorithm to optimize the set of interactions between the ligand and the protein. The binding site was defined as a sphere with a 10.0 Å radius, centered on the Ne atom of residue Gln 257. For each conformer, 20 docking runs were performed, with no early termination, and the GoldScore scoring function with default parameters. Free corners of ligand rings were allowed to flip above or below the plane of their neighboring atoms during docking, and intramolecular hydrogen bonds in the ligand were allowed to form. Torsion angle distributions were from the CSD. A template similarity constraint was added to bias the conformation of docked ligands toward solutions where the acceptor atoms in the ligand were close in space to the acceptor atoms in bound phorbol-13-O-acetate from the crystal structure.

Reaction Energies: Density functional theory (DFT) calculations in Gaussian 09[13] were used for geometry optimizations and for reaction energy calculations. The geometry optimizations and frequency calculations were done using the B97-D3 functional[14,15] with the 6-31G(d) basis set. Tight optimization convergence criteria were used, along with the ultrafine integration grid. Single point energies were calculated with the ωB97X-D functional[16] and the 6-311G(2d,2p) basis set. Both of these functionals include dispersion corrections to long-range

[12] Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
[13] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J. *Gaussian 09*; Gaussian, Inc.: Wallingford, CT, 2009.
[14] Grimme, S. *J. Comput. Chem.* **2006**, *27*, 1787.
[15] Grimme, S.; Ehrlich, S.; Goerigk, L. *J. Comput. Chem.* **2011**, *32*, 1456.
[16] Chai, J.-D.; Head-Gordon, M. *Phys. Chem. Chem. Phys.* **2008**, *10*, 6615.

interactions which have been shown to be essential for accurate isomerization reaction energy calculations, especially in large molecules.[17] The bryostatin analogues examined here are large (>100 atoms, even with the acyl chain truncation) and floppy, and even at an energy minimum retain a number of low-frequency normal modes corresponding to flexing and bending motions of the full macrolide ring. Thus the harmonic oscillator approximation used by Gaussian for the estimation of zero-point energies and thermal contributions to the enthalpy is not really valid for these molecules, and the isomerization reaction energies were estimated using the electronic energies without any correction or scaling factors.

[17] Huenerbein, R.; Schirmer, B.; Moellmann, J.; Grimme, S. *Phys. Chem. Chem. Phys.* **2010**, *12*, 6940.